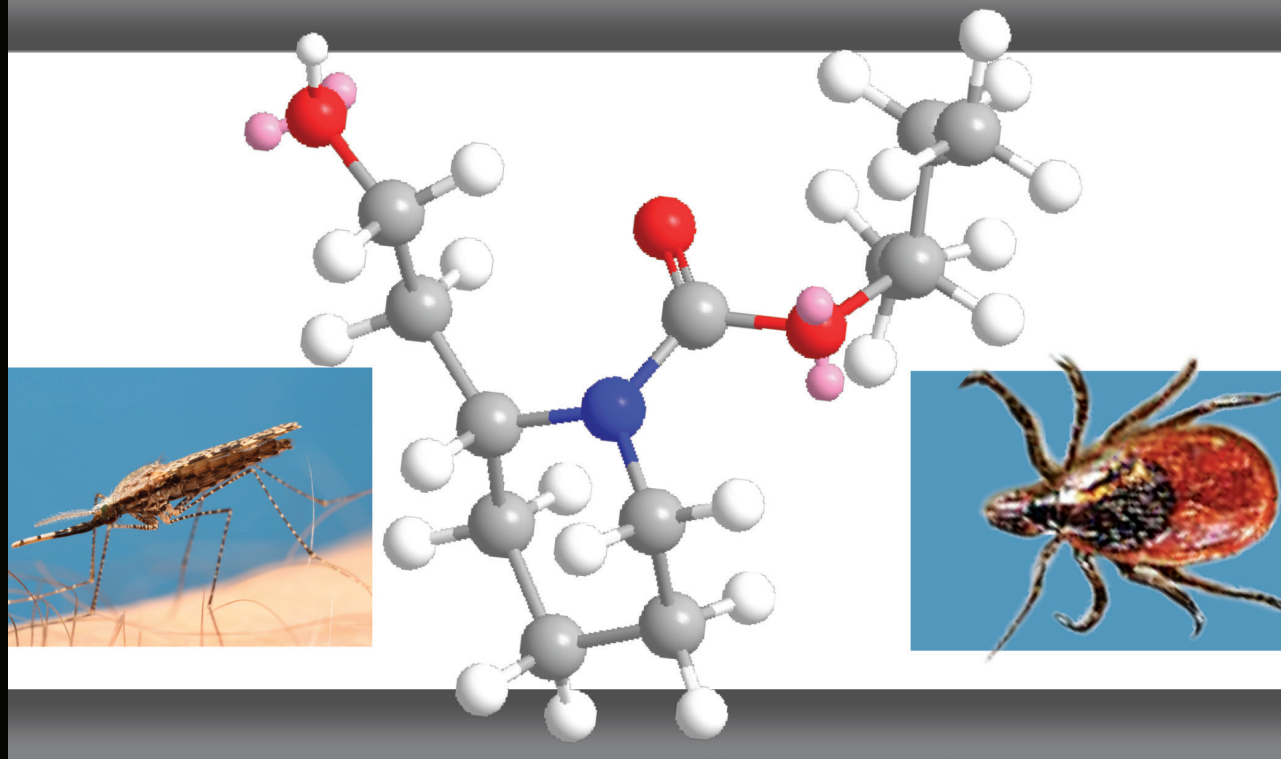
SECOND EDITION

Insect Repellents Handbook



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My dear three brothers and four sisters; my beautiful and loving wife, Natalie, and our wonderful children: Ameena, Adam, and David; and to the memory of my beloved parents.

Mustapha Debboun

This book is dedicated to the memory of Professor Chris Curtis and Dr. Nigel Hill, London School of Tropical Medicine and Hygiene, who spent their working lives developing and testing methods to protect people against vector-borne disease.

Stephen P. Frances

This book is dedicated to the thousands of volunteers who, over the years, have tested and retested repellents. They experienced many hours of discomfort so that others could be spared from insect and tick bites.

Daniel A. Strickman

Contents

Preface

The first edition of this book was *Insect Repellents: Principles, Methods, and Uses.* It was the first comprehensive volume on the subject. Since its publication in 2007, both the science and development of repellents have advanced considerably. What is more, we have observed the kinds of uses readers have made of the first edition and we decided to produce a more focused and accessible volume. For that reason, we changed the title of this second edition to *Insect Repellents Handbook*. It is our intention to reflect the current state of insect repellent knowledge that contains definitive treatments of each major area in the discipline, including the science, the development, and uses of insect repellents. We believe that it will be a reference and resource that is rich in data for professional insect repellent researchers, public health professionals, medical professionals, medical entomologists, vector control professionals, biologists, environmentalists, wildlife professionals, industry and sales personnel, and government regulators. The *Insect Repellents Handbook* will provide a thorough understanding of insect repellent science and practices based on a rigorous and complete treatment of the subject compiled by leading scientists in each topic of insect repellents.

The first section of the handbook summarizes scientific developments about insect repellents. Ranging from new understanding of how repellents work to modern methods for finding new active ingredients, this section should provide an efficient way to catch up with recent progress. The second section of the volume presents detailed treatments of methods for developing, evaluating, and formulating repellents. Finally, the third section discusses how repellents can be used to protect an individual from arthropod bites and associated diseases, as well as possible advancements in the future.

Repellent science and development is at an exciting stage. New understanding of how repellents work promises to deliver new, highly active compounds that may lead to entirely new ways of using insect repellents. The current worldwide emphasis on prevention of infection from vector-borne pathogens has challenged those who study repellents to evaluate how to use repellents for that purpose. New formulations could lead to products that are not only more effective but also more acceptable to the user. Taken together, developments now may lead to completely new and more effective ways to use repellents in the future.

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PART I

Science

CHAPTER 1

Terminology of Insect Repellents

Graham B. White and Sarah J. Moore

CONTENTS

> Smell is fatal for repellents intended to be used in jungle warfare, but, provided it is pleasant, it may even be an advantage in civilian use. Owing to the importance attached to long duration of effectiveness for military purposes, research on repellents during the war has tended to develop a type of repellent with very high boiling-point and hence, almost as a corollary, less effective at a distance than some more volatile repellents.
>
> **(Christophers, 1947)[1]**

BASIC REPELLENT TERMINOLOGY

The English word *repellent* is a noun (the repellent material) or an adjective (repellent effect) derived from the Latin verb *repellere*, meaning "to drive back," the movement away being repulsion.[2–4] The alternative spelling *repellant* with an "a" comes from *antem*, meaning "an agent of action." Attractant has the opposite meaning, based on the Latin *attractum* for being pulled toward something. The word *attractant* is a noun (something that attracts) or an adjective (being attractive) depending on the context and syntax; it is etymologically derived from the Latin verb *trahere*, meaning "to draw or pull." Therefore, anything that attracts or repels particular insects is either an insect attractant or an *insect repellent*. Generally, for chemicals affecting feeding behavior negatively or positively, by any mode of action, the first edition of this book introduced the new term *phagomone*. Some materials and physical factors (e.g., heat and light) can elicit either repellent or attractant effects, depending on quantitative factors (Chapters 2, 4, and 8) and circumstances.

To help foster scientific perceptions, Dethier[5–7] defined repellents as "any stimulus which elicits an avoiding reaction" and made a further distinction, in terms of the physical state of the chemical, by recognizing contact repellents and vapor repellents; meaning those that have to be touched by the insect or simply detected in the air. Differentiating these modes of exposure remains challenging, as

discussed in Part II of this book, because the treatment distinction may not be absolute. Generally, to achieve personal protection with some duration of effectiveness repellents are applied ad libitum to chosen parts of the skin and clothes; due to this topical treatment (derived from the Greek word *topos*, meaning "limited location"), they are commonly known as topical repellents. Some devices, for example, mosquito coils and repellent vaporizers (dispensers and emanators), are designed to protect an outdoor area or an indoor volume of space by releasing vapors that give an area repellent effect[8–10] for as long as the spatial repellent devices operate (Chapter 15), but their effectiveness quickly fades when emission stops and the repellent dissipates.

Commercially, insect repellents are consumer products marketed in every society through suitable retailers (e.g., camping and travel shops, pharmacies, and supermarkets) and by mail order. The traditional repellent business became more scientifically rigorous when synthetic chemicals began to replace botanicals as the products of choice during the 1940s and 1950s. Previously, the so-called culicifuges and repellents to ward off noxious arthropods comprised a wide variety of popular natural products (Chapters 14 and 15), few of which had been evaluated entomologically or standardized for efficacy. The repellent market grew and evolved rapidly following the 1939–1945 World War II period, thanks to the results of intense research efforts to discover and develop repellents for military use, as described in this chapter. Hence, the technical foundations of repellent science were mainly established by three loosely coordinated groups working in Rutgers University,[11,12] New Jersey; Cambridge,[13,14] United Kingdom; and Orlando,[15–18] Florida, continuing to this day at Gainesville,[19,20] Florida. They developed standardized testing methods with mosquitoes (*Aedes aegypti*) and ticks (*Amblyomma americanum*), which still provide the basis of screening procedures and comparative assessment of repellents (Chapter 9).

The following section attempts to explain the meanings of a wide range of terms needed to understand repellent science and associated research. This list and supporting references augment the greater attention given to the major topics in successive chapters of this book. The acronyms for relevant organizations and regulatory statutes are included here. The index of this book provides further references to keywords and Chapters 10, 11, and the Appendix provide chemical designations for many of the active ingredients.

GLOSSARY AND DEFINITIONS FOR REPELLENT SCIENCE

Abbreviations and grammatical types of words are denoted as follows: *adj.* = adjective; *amer.* = American spelling; *cf.* = compare; e.g. = for example; *eng.* = English spelling; *n* = noun; *pl.* = plural; *ref.* = refer to; *sing.* = singular; and *vb* = verb. Bold words have their own entries in this glossary.

2-undecanone: methyl nonyl ketone (CAS number 112-12-9) repellent derived from tomatoes, commercialized as BioUD, affecting mosquito odorant receptors differently[25,89] from **deet** and **picaridin**.

abiotic factors: pertaining to repellents: nonbiological variables that may influence repellency, e.g., air quality, humidity, light, temperature, and wind (*cf.* **biotic factors**).

absorb (*vb*), absorption (*n*), absorptive (*adj.*): process by which something (e.g., repellent) enters a substrate, e.g., skin (*cf.* **adsorb**).

acaricide: chemical agent used to kill ticks and/or mites (Acari); mostly suitable for use also as insecticides (*cf.* pesticides).

acidity: pH < 7.

activator: for a repellent, something (e.g., heat, synergist, and volatile solvent) that when added to or combined with the repellent increases its availability or activity.

active ingredient (a.i.), active material: see **ingredient**.

acute toxicity: rapid short-term expression of toxic (poisonous) effects from exposure to something causing pathological symptoms or death from a single or limited exposure.

additive effects: effects of repellents that, when used in combination, provide the sum of their effects; nonadditive effects of repellents that, when used together, have no more effectiveness than only one of them.

adjuvant: inert chemical added to a repellent formulation to enhance its effectiveness.

ad libitum: at liberty, unlimited, e.g.,[21] "mosquito adults that emerged were fed *ad libitum* on a 10% glucose solution."

adsorb (*vb*), adsorption (*n*), adsorptive (*adj.*): process by which something (e.g., repellent) is bound to the surface of a particle or absorbent substance.

aerosol: extremely fine spray droplets suspended in air. The World Health Organization (WHO)[22] classifies spray droplets as fine aerosols, <25 μm; coarse aerosols, 25–50 μm; mists, 50–100 μm; fine sprays, 100–200 μm; medium sprays, 200–300 μm; and coarse sprays, >300 μm.

affinity: attraction. In chemistry, the tendency of molecules to associate; for an agonist or antagonist, the measurement[3] of affinity is the reciprocal of the equilibrium dissociation constant of the ligand–receptor complex, denoted as K_A, which is calculated as the rate constant for offset (k_{-1}) divided by the rate constant for onset (k_1).

aggregate (*n or vb*): To gather together, assemble.

agonist (*n*), agonism (*vb*): a chemical that binds to a receptor of a cell and triggers a physiological response within that cell. In the case of repellents, this causes behavioral modification through binding to olfactory receptors (ORs), resulting in the stimulation of olfactory receptor neurons (ORNs) or olfactory coreceptors (Orco),[23] resulting in avoidance. The insect repellent *N*,*N*-diethyl-3-methylbenzamide (deet) (Figure 1.1) is an agonist of some insect ORs in the absence of other odors, as reported for mosquito larva *Anopheles gambiae*[24] and adult *Aedes aegypti*.[25] Other agonist repellents that elicit avoidance (repellency) include eucalyptol, linalool, and thujone.[26]

alkalinity: pH > 7.

allelochemicals: nonnutritional semiochemicals used by one species to affect (behavior, feeding, growth, health, and breeding of) another species.

allomone: chemical substance (produced or acquired by an organism) that when contacting an individual of another species evokes in the receiver a behavioral or physiological reaction adaptively favorable to the emitter (opposite of kairomone).

analogs (*amer.*), analogues (*eng.*): similar chemical structures (differing by alternative atoms or equivalent functional groups) with contrasted biological and/or chemical properties.

analytical-grade chemical: purified after synthesis, containing negligible amounts of impurities (*cf.* **technical grade**); useful standard for analytical comparison and research.

anosmia[27] **(*n*), anosmic (*adj.*)**: inability of an organism to perceive an **odor**. Used mainly for inhibition of attractive responses through exposure of insects to an **attraction inhibitor**[28] or **masking agent**[29] and for individuals who no longer respond to an odor (to which they were previously responsive) due to damage induced by pathogens.[21]

antagonist (*n*), antagonism (*n*): a chemical that interferes with the action of an agonist, and blocks or dampens agonist-mediated responses. This reduces the potency of a repellent. Antagonists modulate the effects of agonists, either by binding to the same active site as the agonist (competitive antagonism) or by interacting with the receptor at binding sites not normally involved in the biological regulation of the receptor's activity, thereby indirectly affecting the responsiveness of the receptor to an agonist, or irreversible blocking of agonist-mediated response, depending on the longevity of the antagonist–receptor complex, which, in turn, depends on the nature of the antagonist–receptor binding. Several broad-spectrum insect repellents are known antagonists of insect olfactory receptors, including deet,[30] picaradin,[25] and IR3535.[25] Antagonists of Orco have also been identified.[31]

antenna (*sing.*)**, antennae** (*pl.*): paired sensory appendages on arthropod head.
anthropomorphize (*vb*): viewing things from human perspective.[32]
anthropophagous, anthropophagy: feeding on humans (*cf.* **zoophagy**).
anthropophilic, anthropophily: tendency of hematophagous anthropods to prefer human hosts.
antiattractant[33]: substance with little or no intrinsic repellency but with the property of diminishing the attractiveness of a lure; equivalent to attraction inhibitor.
antibiosis[34,35]: host mechanism to deter pests.
antifeedant: substance that inhibits normal feeding activity (*cf.* **phagostimulant**).
antixenosis[35,36]: phytophagous insects showing nonpreference for a resistant plant compared with a susceptible plant; this term could be applied to host-specific hematophagous arthropods.
AOAC (Association of Official Analytical Chemists) International (http://www.aoac.org): founded in 1884; oversees the most extensive program for the validation of Official Methods of Analysis, but none specifically for repellents (*cf.* **CIPAC**).
aqueous: dilution in water.
area repellent (Chapter 15): equivalent to space repellent, spatial repellency; at higher concentrations, pyrethroid area repellents (allethrins, metofluthrin, and transfluthrin) have knockdown effects and tend to be more insecticidal.
aromatic: generally, a fragrant and pleasant odor; in chemistry, a compound containing at least one ring-type group in its molecule; solvents containing benzene or its derivatives.
arrestant: chemical or physical source (light, heat, etc.) that causes organisms to aggregate in contact with it, by arrestment, the mechanism of aggregation being kinetic (by movement) or having a kinetic component.[7] An arrestant does not attract, but retains organisms once in the vicinity, by reducing the speed of linear locomotion and/or increasing the turning rate (*cf.* locomotor stimulant). The *-ant* form of this word is etymologically correct (not arrestent), being derived from the Latin *arrestare*.
arthropods: invertebrate phylum Arthropoda. Creatures with exoskeleton (consisting of chitin) and jointed legs. Blood-feeding (hematophagous) arthropods are either insects (class Insecta) or mites/ticks (class Arachnida, order Acari). Numerous other groups of animals affect humans directly through bites or envenomation (e.g., snakes, scorpions, spiders, and wasps). Repellents may be useful against all these biters.
attractant: for insects, something that causes attraction, whereby the attracted insects make oriented movements toward its source,[7] that is, the opposite of repellent (Chapter 9); useful to lure for trapping.[37] Commonly employed attractants[38–40] for hematophagous arthropods include acetone, carbon dioxide, 1-octen-3-ol, and lactic acid. Associated terms: to attract (*vb*); attractance (*n*), the quality of attracting; attraction (*n*), the act of attracting or the state of being attracted; and attractive (*adj.*), serving to attract. Oviposition attractant serves for gravid females to locate suitable oviposition sites, e.g., *erythro*-6-acetoxy-5-hexadecanolide[41,42] for *Culex quinquefasciatus*. Sex attractant is a substance or a mixture of substances released by an organism to attract members of the opposite sex of the same species for mating.
attraction inhibitor: compounds with an effect that results in a reduction[43] of the number of organisms that respond to an attractive stimulus. In the case of insects, this is accomplished[44] by inhibition or excitation of olfactory receptor neuron (ORN) responses.
avoidance: movement away from something; *ref.* deterrence, negative taxis, and excitorepellency (Chapter 6).
behavioristic avoidance[45]: also known as behavioral[46] (*Amer.*) or behavioural (*Eng.*) avoidance, or behavioristic resistance or protective avoidance—modified behavior whereby endophilic mosquito populations sometimes adapt to exophily in response to the pressure of indoor residual spraying with an excitorepellent **insecticide**, or community use of insecticide-treated bednets.

bioassays: standard methods and procedures for replicated comparative testing of effects on biological materials.[47–49] Chapters 4, 6, 8 and 14 describe bioassays for attractants and repellents.

Biocidal Products Directive of the **European Commission**: regulatory law for pesticides in all countries of the European Union, implemented by national governments and the European Union Environment Directorate (http://europa.eu.int/comm/environment/biocides/index.htm). This Directive 98/8/EC of the European Parliament and of the council on the placing on the market of biocidal products was adopted in 1998. According to this directive, member states had to transpose the rules before May 14, 2000 into national law. The Biocidal Product Directive aims to harmonize the European market for biocidal products and their active substances. At the same time, it aims to provide a high level of protection for humans, animals, and the environment. The commission adopted the original proposal for the Biocidal Products Directive in 1993, following the model established by Directive 91/414/EEC on plant protection products adopted in 1991.

biocide: chemical that has a wide range of toxic properties, usually to both plants and animals.

biotic factors: pertaining to repellents. Biological variables that may influence repellency, such as physiological condition of the **insect** (e.g., level of hunger and activity cycle) or the host (e.g., rates of exhalation and sweating) (*cf.* **abiotic factors**).

bite, biting[51] (*vb.*): **hematophagous** act of penetrating skin by the mouthparts of an insect or other arthropod with ingestion of blood (*cf.* **probe**, **landing**, **questing**).

biting midges, no-see-ums, punkies, American sand flies: a family of very small but painful biting flies (Diptera: Ceratopogonidae) with more than 4000 species described; the most conspicuous genera are *Culicoides* and *Leptoconops*.

biting rate: the number of bites per person per time period (e.g., 12 bites/h), as a measure of population density in relation to humans, for any given species of biting arthropods or group of species at a particular place and time. For ethical reasons, especially where vector-borne disease risks must be considered, it is customary to intercept the attacking insects before they actually bite (possibly increasing catch efficiency); the results are therefore reported in terms of the landing rate rather than the **biting rate**. The coefficient of protection[50] (CP) is given by $[(A - B)/A] \times 100$, where A is the average number biting the untreated person per hour and B is the average number biting the experimentally treated subject during the same exposure period and under the same conditions; CP is commonly used to assess the relative effectiveness of candidate materials compared to deet. Other criteria for repellent testing under field conditions are the period of time to first bite (TFB), or first confirmed bite, or duration of a reduction in biting, the choice of criterion depending *inter alia* on the local **biting rate** pressure.[51] Considerable disagreement exists on the appropriate measurement of repellent product efficacy, as discussed throughout this book.

blackflies: a family of very painful bloodsucking flies (**Diptera**: Simuliidae) with more than 2000 species described, the main worldwide genus being *Simulium*.

botanical: pertaining to green plants (Embryophytes); plant sources of repellent natural products.

butyl carbitol acetate: also known as diethylene glycol monobutyl ether acetate. This compound was the standard of comparison adopted by Granett[11,12] for screening repellents during the 1940s at Rutgers University, and the Orlando Laboratory of the Bureau of Entomology, precursor of the Insects Affecting Man and Animals Research Laboratory at Gainesville, now the Center for Medical, Agricultural and Veterinary Entomology, of the Agricultural Research Service (ARS) of the U.S. Department of Agriculture (**USDA**).

CAFIK: Continuous Action Flying Insect Killer.

carrier: inert solid or liquid material used to prepare a repellent formulation.

CAS numbers: unique numerical identifiers for chemical compounds, polymers, mixtures, and biological sequences. Chemical Abstracts Service (CAS), a division of the American Chemical

Society, assigns these identifiers to every chemical described in the literature. They are also called CAS registry numbers (CAS RNs). Substances also receive unique CA index names, constructed using rigid nomenclature rules. In an effort to facilitate searching for related compounds, the most important functional groups of a substance are named first, followed by their modifications (*cf.* **IUPAC** names) (http://www.cas.org/EO/regsys.html).

CDC (Centers for Disease Control and Prevention): U.S. Department of Health and Human Services. CDC policy and guidelines[52,53] for repellents are issued by the Division of Vector-Borne Infectious Diseases and implemented by the National Center for Infectious Diseases (based at Fort Collins, Colorado) and by the Entomology Branch (based at Atlanta, Georgia).

CFR (Code of Federal Regulations) of the United States (http://www.gpoaccess.gov/cfr/index.html): concerning pesticides, including repellents. Title 21 deals with Food and Drug Administration (**FDA**), including Generally Regarded as Safe (**GRAS)** materials, and Title 40 deals with Environmental Protection Agency (**EPA**) including the Federal Insecticide, Fungicide, and Rodenticide Act (**FIFRA**) and the Food Quality Protection Act (**FQPA**) (Chapter 17).

channel: *ref.* **ion channel**.

chemical: an element or a compound (*n*); pertaining to chemistry (*adj.*).

chemistry: chemical properties and the science of dealing with them.

chemoreceptor: insects rely on their olfactory systems, which express a large number of chemoreceptors that belong to three main classes: **ionotropic receptors** (IRs), **gustatory** receptors (GRs), and **odorant receptors** (ORs), to locate food, oviposition sites, mates and to avoid danger. Insect ORs (structurally and genetically unrelated to vertebrate receptors) consist of two distinct molecules[54] serving as receptor for the odorant and as an ion channel that is gated by the binding of the odorant.

chiggers: biting mite larvae (family Trombiculidae, order Acari, class Arachnica) and adult jigger fleas (family Tungidae, order Siphonaptera, class Insecta) that burrow into mammalian skin and swell to form chigoes.

CIPAC (Collaborative International Pesticides Analytical Council) (http://www.cipac.org): a recognized international, nonprofit, and nongovernmental organization, promotes international agreement on methods for the analysis of **pesticides** and physicochemical test methods for formulations. Methods are proposed by manufacturers (companies) and tested internationally by the interlaboratory program for evaluation of test methods. After validation of analytical results and adoption, the methods are published in CIPAC handbooks.

compatible ingredients: ingredients that retain their individual properties when mixed together.

complete protection time[51,127] (CPT): amount of time from application of repellent until efficacy failure, as defined in each study, usually the first mosquito landing and/or probing (Chapter 8); *ref.* **repellency duration**.

compound (*n or vb*): chemical material of more than one element combined into a substance having its own properties, differing from those of its constituents (*cf.* **mixture** or **formulation** of ingredients that retain different properties).

concentrate (*n or vb*): chemical formulation containing a high percentage of **active ingredient (a.i.)**.

concentration (*n*): proportion of a given ingredient in a formulation or solution, e.g., ounces per gallon and milligrams per liter.

confusant[55] (*n*): a molecule that alters the way that **olfactory receptors** react to a given chemical, so insects are still capable of detecting odors but don't respond normally to them.

control: biologically, to suppress (*cf.* **eliminate, eradicate**); experimentally, to serve as the untreated sample for comparison with the treated sample.

cosmetic: serving to beautify (*adj.*), or a preparation for beautifying (*n*) the face, hair, skin, and so on. Chapter VI of the U.S. Federal Food, Drug, and Cosmetic Act of 1906, Title 21 of the U.S. Code, plus amendments (currently administered by the FDA), defines cosmetics as articles intended to be applied to the human body for cleansing, beautifying, and

promoting attractiveness or altering the appearance without affecting the body's structure or functions. Products such as skin creams, lotions, perfumes, lipsticks, fingernail polishes, eye and facial makeup preparations, shampoos, permanent waves, hair colors, toothpastes, deodorants, and any material intended for use as a component of a cosmetic product are included in this definition. Soap products consisting primarily of an alkali salt of a fatty acid and making no label claim other than cleansing the human body are not considered cosmetics under U.S. law. Likewise, insect repellents are not cosmetic products, although it would be possible to include repellent active ingredients in particular cosmetics as done with some *sun screen* anti-UV preparations combined with **deet** (that enhances absorption, raising systemic toxicity)[56] marketed for giving skin protection against both sunburn and biting insects. The term *cosmetic properties* of a repellent product is often used to describe the properties of the formulation that do not affect performance but alter the subjective perception of the product (e.g., fragrance, oiliness, and color).

CSPA (Consumer Specialty Products Association) (http://www.cspa.org): represents the interests of the consumer specialty products industry in the United States, providing households, institutions, and industrial customers with products for a cleaner and healthier environment.

CTFA (Cosmetic, Toiletry, and Fragrance Association) (http://www.ctfa.org): publisher of the *International Cosmetic Ingredient Dictionary and Handbook*,[57] which gives International Nomenclature Cosmetic Ingredient (INCI) names for cosmetics and personal care products, e.g., ethyl butyl acetyl aminopropionate (EBAAP) for IR3535.

cue: signal for an action.

culicifuge[58,59]: repellent for use against **mosquitoes** (Culicidae); the suffix is based on the Latin verb *fugere*, meaning "to flee."

cuticle (*n*), cuticular (*adj.*): **arthropod** exoskeleton material, formed of several layers containing chitin, wax, and proteins secreted by hypodermal cells.

DDT: dichlorodiphenyltrichloroethane insecticide.

deet: *N*,*N*-diethyl-3-methylbenzamide (originally known as *N*,*N*-diethyl-*meta*-toluamide) (Figure 1.1), usually abbreviated to deet or DEET in the literature. It is the dominant repellent used worldwide since the 1960s. Globally, it is the leading active ingredient of insect-repellent products, being effective against all groups of biting arthropods and even leeches. Formulations containing from 4% to 100% deet are registered by the EPA for direct skin application to repel insects, rather than killing them. Deet is registered for use by consumers, plus a few veterinary uses, but not for food treatment. It was approved by the World Health Organization Pesticides Evaluation Scheme (WHOPES), but obsolete specifications were withdrawn in 2009. Market surveys in the United States show that about a third of the population uses deet-based products, currently available to the public in a variety of liquids, lotions, sprays, and impregnated materials (e.g., wipes and wristbands). After it was discovered by the USDA ARS and developed by the U.S. Army in 1946, deet was introduced for use by the general public in 1957. More than 230 products containing deet (CAS number 134-62-3) are currently registered with the EPA by more than 70 companies (http://deet.com and http://www.deetonline.org). Further details on deet are given in Chapters 14, 18, and 20.

deterrent (*n or adj.*): in the repellent context, something that inhibits feeding or oviposition when present in a place where insects would, in its absence, feed, rest, or oviposit.[7] In the biological context, something that protects[60,61] against bodily harm. Associated terms include **deter** (*vb*), to discourage or prevent, and **deterrence** (*n*), the act of deterring. These terms fit the way that permethrin-impregnated materials (e.g., clothes or bednets) and space repellents deter bloodthirsty female mosquitoes from biting or even from entering a house, whereas other pyrethroid treatments are more insecticidal than deterring or repelling (*cf.* **excitorepellent**).

diffuse (*vb*): to disperse, scatter, or spread an idea, vapor, liquid, or solution (not defuse [sometimes misused for repellent diffusion], which means to remove the ignition fuse from something explosive or the fuse-wire from an electric circuit).

diluent: material used to reduce the concentration of an active ingredient in a formulation, e.g., dilution of a concentrate to make the operational concentration.

dimethyl phthalate (DMP) (CAS number 131-11-3): an insect repellent with many other uses as a plasticizer and in solid rocket propellants. Commercially, DMP is superseded by deet, *N,N*-diethyl phenylacetamide, *p*-menthane-3,8-diol (PMD), and others (Chapter 14) for repellent markets.

Diptera: the insect order Diptera (recognized by Aristotle in the fourth century BC) includes approximately 150 families of two-winged flies, true flies. **Biting flies** (~11,000 species) mostly belong to the families/subfamilies Ceratopogonidae (biting midges or no-see-ums), Culicidae (mosquitoes), Glossinidae (tsetse), Phlebotominae (sand flies), Simuliidae (blackflies), Stomoxyinae (stable flies), and Tabanidae (deerflies and horseflies).

disengagent[62] (*n*): a chemical that reduces the interaction between an organism and the source of stimulation, but it is unknown whether the chemical operates as a repellent or by some undirected means such as inhibition.

dispenser or **emanator** for **spatial repellent** vapor: disseminated by heat or passive[63] vaporization.

dispersing agent: material that reduces the attraction between particles.

divert (*vb*)**, diversion** (*n*): movement of a hematophagous arthropod from a protected to an unprotected target[64] caused by the use of repellents. In repellent testing methodology, it is important that repellents are tested in noncompetitive assays to prevent diversion of **mosquitoes** onto controls that would result in overestimation of the efficacy of a **repellent**.

dose: quantity applied at one time.

dosage: quantity of active ingredient applied per unit of time (e.g., 10 oz/day) or area (1 ml/m^2) or volume (e.g., 1 mg/L) or personal application (e.g., 1 ml/arm/day). See Chapters 4, 8, and 14 for dosage criteria employed for comparative evaluation of **repellents**, including the effective dose (actual concentration) giving 50% (median effective dose) or 90% reduction of biting (ED_{50} and ED_{90}) and the minimum effective dose to prevent biting completely; these **bioassay** parameters are mostly employed for comparative studies in the laboratory; *ref.* biting rate for field criteria, discussed in Chapters 9, 18, and 19.

duration of repellency: see **repellency duration**.

EAG: **electroantennogram**.

EBAAP (ethyl butyl acetyl aminopropionate) (INCI name): **CAS number** 52304-36-6; chemical description 3-(*N*-*n*-butyl-*N*-acetyl)-aminopropionic acid, ethyl ester; derived synthetically from β-alanine (a natural amino acid); commercially known as IR3535®. Approved by the **WHOPES**[66] and specifications issued in 2006.

ECB (European Chemicals Bureau): responsible inter alia for the Biocidal Products Directive of the European Commission (http://ecb.jrc.it/biocides/).

EDTIAR: extended Duration Topical Insect and Arthropod Repellent (deet-based slow-release formulation) introduced in 1990 for U.S. military use; commercially marketed as Ultrathon™ (http://www.ultrathon.com), registered trademark of 3M Corporation, St. Paul, Minnesota.

effective concentration (EC_{50}, EC_{90}): in repellent testing methodology, the concentration of repellent that is sufficient to prevent 50% and 90% of attempted **mosquito** bites, respectively. EC_{50} is usually calculated from a graded dose–response curve after a specified exposure duration.[67] PUF sampling method[68] can be used to assess repellent concentration in air causing spatial repellency against mosquitoes (Chapter 12).

effective dose (ED_{50}, ED_{90}): combines effective concentration with duration of exposure.

effectiveness[69]: actually producing the intended or expected result (*cf.* **efficacy**).

efficacy[69]: capacity to produce an effect (*cf.* **effectiveness**).

EIR: **entomological inoculation rate**.

electroantennogram (EAG): measurement of electrical activity in the antenna, employed[21,70] to identify attractants and repellents.

electropalpogram (EPG): measurement of electrical activity in the maxillary palp, employed[21] to identify attractants and repellents.

eliminate[71]: to remove or expel from a defined situation (*cf.* **control**, **eradicate**), e.g., to eliminate mosquitoes from a house.

emanator or **dispenser** for **spatial repellent** vapor: disseminated by heat or passive[64] vaporization.

emulsion: mixture consisting of minute globules of one liquid suspended within another liquid, e.g., oil in water. An important component is the surfactant emulsifier (e.g., agar and lecithins), partly hydrophilic and partly lipophilic, promoting the suspension of one liquid in another.

endophagic (*adj.*)**, endophagy** (*n*): feeding indoors by endophilic mosquitoes, and so on.

endophilic (*adj.*)**, endophily** (*n*): tendency of insects (especially female *Anopheles* mosquitoes of some species) to come into houses for biting nocturnally and resting diurnally (opposite of exophily).

engagent[63]: a chemical that increases interaction of an organism with the source of stimulation, but it is not yet known whether that end result is mediated by arrestment or attraction.

entomological inoculation rate (EIR): mosquito biting rate (bites per person per year) × proportion of infected mosquitoes (infectivity rate).

entomology: the study of insects; commonly assumed to include other **arthropods**.

entopomorphize (*vb*): viewing things from the insect's perspective (*cf.* **anthropomorphize** and **phytopomorphize**).

EPA: Environmental Protection Agency of the United States (USEPA), regulatory agency responsible for repellents in the United States, through two main acts: **FIFRA** and **FQPA**.

EPG: **electropalpogram**

eradicate[71]: to completely remove or destroy a species or disease so that it cannot return (*cf.* **control, eliminate**) because that type of organism is extinct.

essential oils: alkaloids, phenols, terpenes, terpenoids, and other volatiles obtained from plants (Chapters 5, 9, and 10) usually by steam distillation or pressing; they are hydrophobic and mostly aromatic.[32] Many are repellent[72–74] to insects, and some are potent insecticides[75–77] traditionally employed as pesticides around the world. Encouraged by the EPA's 1996 exemption to FIFRA for minimum risk pesticides, many have recently been developed and commercialized as so-called exempt **pesticides** in the United States. Among the ones most effective[78] as repellents are white cedar oil (CAS number 8000-34-8), peppermint oil (CAS number 806-90-4), red thyme oil (CAS number 8007-46-3), bourbon geranium oil (CAS number 8000-46-2), linalool, and dehydrolinalool.[79] However, as indicated in Chapter 12, toxicological risk assessment is necessary to establish safety and tolerance levels for essential oils used as repellents or in foodstuffs (FDA category [CFR 21:170] **GRAS**).

EU: the European Union of 28 countries (2013) with 20 official languages, formerly known as the European Community (EC) and originally the European Economic Community. The Biocidal Products Directive and Registration, Evaluation, Authorisation and Restriction of Chemical substances (REACH) determine pesticide regulatory status throughout the EU (http://europa.eu.int/).

evaporate: to change from solid or liquid to vapor (*amer.*) or vapour (*eng.*), synonymous with vaporize (*amer.*) or vapourise (*eng.*); evaporation (*n*): the process of evaporating; evaporate to dryness.

excitorepellency (*n*): the power of (**DDT**) and other irritant compounds, notably certain **pyrethroids**, usually through tarsal exposure, to stimulate insect activity so that they take off before **knockdown**, even from sublethal exposure[45,80–82]; thereby, adult female mosquitoes become

more **exophilic** instead of **endophilic** and this contributes to a greater reduction of their **vectorial capacity**[83,84] than from simply killing a lesser proportion of the **vector** population. There is no need for a hyphen in the middle of "excito-repellency."

excitorepellent (*n or adj.*): chemical causing insects to make undirected movements that set them apart from the source,[7] due to undirectional combination of orthokinesis (speed changes) and klinokinesis (turning changes), proportional to the stimulus intensity (Chapter 6). When exposed to excitorepellent insecticides,[46] **mosquitoes** tend to move toward light, resulting in their escape from treated houses.

exempt (*n or vb*): generally means *taken out*; but for U.S. legal purposes, FIFRA Section 25b lists certain pesticides as being exempt from EPA registration (www.epa.gov/oppbppd1/biopesticides/regtools/25b_list.htm) including the following repellent oils: castor, cedar, cinnamon, citronella, clove, eugenol, garlic, geraniol, geranium, lemongrass, linseed, mint, peppermint, 2-phenethyl propionate (from peanuts), rosemary, sesame, soybean, thyme, and white pepper.

exophagous (*adj.*)**, exophagy** (*n*): behavioral tendency of female mosquitoes, and so on to bite hosts outdoors.

exophilic (*adj.*)**, exophily** (*n*): tendency of most insects to stay outside buildings (contrasts with endophily for malaria vector *Anopheles* females that enter houses to bite and take shelter).

expel: to drive out, as when endophilic mosquitoes leave a house following indoor exposure to a repellent or an **excitorepellent**.

FDA (http://www.fda.com/): the Food and Drug Administration of the U.S. Department of Health and Human Services, having regulatory responsibility for cosmetics and medicines, and so on, but not for insect repellents.

FIFRA: the U.S. Federal Insecticide, Fungicide, and Rodenticide Act (1947, 1972, and amendments) for pesticides regulation (40 CFR), administered by the **EPA**.

flies: **Diptera**, two-winged flies (one pair of wings), including mosquitoes (family Culicidae).

formulation: defined chemical mixture, usually meaning the commercialized version of a special formula; most formulations require dilution for use.

FQPA: Food Quality Protection Act, U.S. Public Law 104–170, 1996 (http://www.epa.gov/oppfead1/fqpa/backgrnd.htm): augmenting **FIFRA**, administered by the **USEPA**; intensifies regulatory controls on pesticides for reasons of human and environmental health and summarizes EPA's role under Title 40, parts 150–189, of the U.S. CFR.

Generally Regarded as Safe (GRAS): classification by the FDA (www.cfsan.fda.gov/~lrd/cfr17030.html), similar to minimum risk classification by the EPA (Chapters 5, 9, 13 and 19).

genus (*sing.*)**, genera** (*pl.*): named group(s) of closely related **species**, e.g., *Aedes* or *Anopheles* mosquitoes (**Diptera**: Culicidae).

GRAS: **Generally Regarded as Safe** (*ref.* **FDA** status).

Good Field Practice and **Good Laboratory Practice (GFP** and **GLP)**: internationally recognized standards of conduct and procedure administered by the Organisation for Economic Co-operation and Development (OECD) to ensure the generation of high-quality and reliable test data related to the safety of industrial chemical substances and preparations in the framework of harmonizing testing procedures for Mutual Acceptance of Data (www.oecd.org/document/63/0,2340,en_2649_34381_2346175_1_1_1_1,00.html).

GRAS: **Generally Regarded as Safe** (*ref.* **FDA** status).

green: color of light near the middle of the rainbow (optical spectrum) where the human eye has maximum sensitivity at around 555 nm (540 THz); popular culture and business have adopted the word green to symbolize "safe = low risk" and **organic**[85,86] following the agricultural green revolution during recent decades. Appropriately limited to **natural products**, such as **essential oils** from plants (Chapters 5, 9, and 10).

gustatory: relating to the sense of taste; gustatory receptor (GR), sensitive to deet (Chapter 2) and its avoidance.[152]

haematophagous (*eng.*), **hematophagous** (*amer.*) **arthropods**: blood-feeding and bloodsucking arthropods—**insects**, **ticks**, and mites; commonly referred to as biting insects.

hazard: potential source of harm. For repellents and other pesticides, the WHO classification[87] based on rat LD_{50} by weight, following oral or dermal exposure, assuming solids are fourfold more hazardous than liquids, recognizes the following categories: class Ia, extremely hazardous; class Ib, highly hazardous; class II, moderately hazardous (e.g., **DDT**, permethrin, and **pyrethrins**); class III, slightly hazardous (e.g., **deet**); plus active ingredients unlikely to cause acute hazard in normal use.

homologs (*amer.*), **homologues** (*eng.*): evolutionarily related chemical structures (e.g., polypeptides) differing by molecular substitutions between individuals (mutations), populations, species, and so on.

host–nonhost discrimination[88]: the process whereby hematophagous arthropods and other ectoparasites may choose between favored and disfavored hosts; **deterrents** and **repellents** may protect the would-be favored host.

hydrogen ion concentration: usually expressed as the negative log (**pH**), a measure of acidity–alkalinity.

immiscible: liquids that cannot mix to form a homogeneous solution.

INCI: International Nomenclature Cosmetic Ingredient names; *ref.* **CTFA**.

incompatible ingredients: ingredients that do not retain their individual properties when mixed together.

ingredient: that which goes into a **compound**, a **formulation**, a **mixture**, or some other preparation; **active ingredient** (**a.i**.), the key ingredient with intended activity; inert ingredients for pesticide formulations permitted by the EPA are listed at www.epa.gov/opprd001/inerts/section25b_inerts.pdf.

inhibition: as discussed in Chapters 2 and 3, activity inhibitors cause a neutral reaction, neither attraction nor repulsion, whereby an insect fails to proceed questing purposefully but is not anesthetized nor narcotized. Dogan and Rossignol[90] describe an **olfactometer** for discriminating between **attraction**, **inhibition,** and **repellency** in mosquitoes.

inhibitor, inhibition: an inhibitor is a compound that suppresses the action with another **phagomone**. Several repellents have been shown[28] to suppress insects' attraction to a resource. This mode of action has been demonstrated to function by inhibiting odorant-evoked currents[30,89] mediated by **odorant receptors (ORs)**.

insect: any individual or species of the arthropod class Insecta. Almost a million species of insects have been scientifically recognized. The word insect derives from the Latin *insectum* for having been cut, referring to the articulated body. Adult insects typically have three pairs of legs (hexapod). Main groups of insects comprise about 30 orders, with the order **Diptera** (two-winged flies) including several families with hematophagous biting habits, such as Ceratopogonidae (biting midges or no-see-ums), Culicidae (**mosquitoes**), Glossinidae (tsetse), Simuliidae (blackflies), Stomoxyinae (stable flies), and Tabanidae (deerflies, horseflies). Repellents may be useful against these biting flies and other blood-feeding bugs (order Hemiptera) such as bedbugs (Cimicidae) and kissing bugs (Triatominae).

insecticide: chemical agent used to kill insects; mostly suitable for use also as acaricides (*cf.* **pesticides**).

insoluble: inability of a substance to dissolve in a particular liquid solvent.

ion channels: are **ionotropic receptors (IRs)** on excitable cells that conduct electrical signals more rapidly than chemical signals, e.g., in response to repellents, semiochemicals, or pesticides that target such receptors (notably, acetylcholinesterase inhibitor pyrethroids).

ionotropic receptors (IRs): open **ion channels** when activated by ionized chemicals to affect cell activity.

IR3535®: commercial name for **EBAAP**, insect repellent.

IR-4: USDA Inter-Regional Project number 4 (http://ir4.rutgers.edu) based at Rutgers University for facilitating **USEPA** and state registrations of sustainable pest management technology for specialty crops and minor uses, including **Public Health Pesticides** and their applications.

irritancy (*n*)**, irritate** (*vb*)**, irritation** (*n*): the power of some chemicals, notably DDT and certain **pyrethroids** (especially those with α-cyano moiety), to activate **arthropods**, causing **excitorepellency** (Chapter 6), usually due to tarsal contact with insecticide-treated surfaces—*contact irritancy*—or from airborne exposure[91]—*noncontact irritancy.*

isomerism: refers to the structural alternatives of chemical molecules. The word isomer (*n*) segments "iso" and "mer" derive from the Greek for equal part. Variants of any chemical compound with the same molecular formula may have different structural formulas (*pl. eng.*), formulae (*pl*. Latin). Depending on the overall structure of isomeric (*adj.*) compounds, they are classified as stereoisomers (spatial isomerism), enantiomers, or geometrical isomers. Stereochemistry is the study of these physical structures and interpretation of their different properties. The simplest isomerism is for a compound to exist in two equivalent forms, known as *cis* and *trans* isomers, forming mirror images of each other. This optical isomerism is commonly called enantiomerism. The so-called racemic mixture contains 50:50 *cis*:*trans*; but properties of *cis* and *trans* are seldom the same, so the synthesis may be designed to favor more *cis* or more *trans* for reasons of cost versus benefit. For instance, *cis*-permethrin is more insecticidal and repellent than *trans*-permethrin, so this pyrethroid is more potent as a pesticide with a higher proportion of *cis* to *trans* (*cis* > *trans*); unfortunately, this correlates with mammalian toxicity, so lower proportions of *cis* to *trans* (*cis* < *trans*) are less hazardous. When synthesized economically for commercial purposes, the so-called technical-grade permethrin is usually supplied with *cis* to *trans* ratios in the range from 25:75 (so-called medical permethrin, being *safer* for humans) to 80:20 (more toxic). Insecticide resistance may be isomer specific, depending on the relative *cis*:*trans* selection pressure and mechanism of resistance. Chiral compounds consist of matching isomers that are not mirror images of each other, like our right and left hands, so they are called dextro and laevo isomers (from Latin terms for right and left) or *Recto* and *Sinistro* isomers (*R* and *S*). These descriptors are applied to each of several chiral centers of heterocyclic molecules. Observing the optical activity of isomers with polarized light, the (-) isomer rotates a light beam counterclockwise (levorotatory), whereas the (+) isomer rotates light clockwise (dextrorotatory). Isomerism of pesticides is generally correlated with differential potency of alternative isomers.

IUPAC (International Union of Pure and Applied Chemistry) (http://www.iupac.org): an international nonprofit, nongovernmental organization for the advancement of chemistry, consisting of national chemistry societies. IUPAC is the recognized authority in developing standards for naming chemical elements and their compounds, through its Interdivisional Committee on Terminology, Nomenclature and Symbols (IUPAC nomenclature) (*cf.* **CAS, CIPAC**).

kairomone (*n*): a substance released by one species that benefits the members of another (e.g., parasites detect host kairomones) by being a signal or **attractant** to them (opposite of **allomone**).

kdr: knock-down resistance gene.

kinesis (*n*)**, kinetic** (*adj.*): nonoriented movement of an organism (*cf.* **taxis** [oriented movement]).

klinokinesis (*n*): change in the rate of turning of an organism, depending on the intensity of the stimulus.

knockdown (KD): sublethal incapacitation; early symptom of an insect responding to a pesticide; not necessarily lethal because metabolic recovery may occur (*cf.* Knockdown resistance, ***Kdr***. Hence, the rates of knockdown and **mortality** are scored separately, usually 1 hour and 24 hours after treatment in bioassays. Knockdown has another meaning in molecular biology, for gene incapacitation by siRNA.

landing[51]: settling on potential host without **biting** or **probing**.

Liquid Electric Dispenser (LED): *ref.* **space repellent.**

locomotor stimulant: a chemical that causes, by a **kinetic** mechanism, insects to disperse from a region more rapidly than if the area did not contain the chemical. The effect may be to increase the speed of locomotion, to cause the insects to carry out avoiding reactions, or to decrease the rate of turning.[92]

lure (*n or vb*): an attractant bait for enticing and trapping, e.g., acetone, carbon dioxide, chickens, lactic acid, and 1-octen-3-ol are commonly used as lures[37,38,40,93,94] for tsetse and mosquitoes.

market value: globally and locally, the price of repellent products is determined by market forces, whereas the sale cost (over-the-counter price) of each repellent unit (pack) includes the values of active ingredients, formulation ingredients, manufacturing and labor, packaging, distribution, promotion, sales and profit margins, plus taxes and tariffs. Worldwide, the global market value[95] of repellents was estimated at $2 billion in 2002.

masking agent[29]: obsolete term for **attraction inhibitor**; *ref.* Chapters 2 and 21.

MED: minimum effective dose; *ref.* **dosage**.

metarchon[96,97]: seldom used term for nontoxic alternative pest **control agents**, such as **confusants**, oviposition attractants, sex attractants, and other behaviorally aversive chemical or other influences.

microencapsulation: aqueous formulation process whereby tiny particles or droplets are surrounded by a coating to create small capsules with diameters between a few micrometers and a few millimeters; microencapsulated formulations may prolong the life of repellents[98] or insecticides applied to skin or clothing by shielding them from the effects of sunlight and moisture while slowing the rate of release of repellents to prolong their efficacy. The release of the functional agent occurs by diffusion through the capsule wall and/or rupture of the microcapsules.

mixture: the product of combining ingredients (such as a **formulation**) that retain their different properties instead of becoming a **compound**.

mode of action (MOA): of a **pesticide**; affects specific molecular target sites with biochemical sequelae in an exposed **arthropod**, causing avoidance behavior due to repellents, pathology, and death from acaricides and insecticides. MOAs of **pesticides** are classified and managed by the Insecticide Resistance Action Committee (www.irac-online.org/teams/mode-of-action/).

mortality rate: proportion of sample killed in a test (usually scored 24 hours after treatment) by exposure to a lethal dose causing fatality; those surviving the treatment have experienced only a sublethal dose that may affect their bionomics and behavior, e.g., **inhibition**, **deterrence**, and **repulsion**.

mosquito (*sing.*), **mosquitoes** (*pl.*): a family of biting flies (**Diptera**: Culicidae) with more than 3500 species described (www.mosquitocatalog.org).

mosquito coil[64]: burnable paste coil with **pesticide** for vaporization to repel and kill mosquitoes; see **space repellent**.

natural products: exploitable materials formed by nature, including foodstuffs and natural fibers used for weaving fabric, e.g., cotton. Repellent products from plants botanicals are reviewed in Chapters 5, 12, 13, 14, 20, and 21; those from nonwoody plants are herbal that is: from herbs. Azadirachtin, **essential oils**, and **pyrethrins** are important as both natural **insecticides** and repellents.

odor (*amer.*), **odour** (*eng.*): airborne chemical(s) capable of activating one or more **ORs** that sense **smell**.

odorant[99]: airborne molecules giving rise to an **odor** sensation; chemical signals or cues such as **semiochemicals** emitted by one organism and perceived by another when they activate **ORs** resulting in a signal.

odorant binding proteins (OBPs): mediate **odor** recognition by **ORs** and may be targeted by **repellents**.[100]

olfactometer: apparatus for measuring the behavioral preferences of arthropods when affected by attractants, odorants, repellents, and other vapors, although the arthropods are given a choice between streams of air with different odors flowing down a Y-tube[79] or multiport[101] olfactometer.

olfactory: pertaining to olfaction,[102] the sense of **smell**.

odorant receptor co-receptor (Orco)[103]: previously Or83b in the fruit fly *Drosophila melanogaster* and OrX in other insect **species**. Insects use ligand-gated ion channels for olfactory signal transduction and Orco functions as an obligate coreceptor with ligand-selective[104] ORs to form a heteromeric[105,144] complex. Relatively little is known about their functional domains or the mechanisms by which odors activate the OR-Orco complex and how ions permeate[144] it. However, it is known that Orco is critical for OR olfactory signaling, as conventional ORs are nonfunctional when expressed[107] without Orco. Orco functionality is required for OR-mediated chemoreception across all insects[108] and enhances odorant responsiveness without altering ligand specificity when coexpressed[145] with ORs. Chapter 2 describes the potential importance of compounds that interfere with Orco and disrupt[23] insect responses to olfactory cues. Targeting Orco provides new scope for broad-spectrum insect specific **confusants**.

olfactory receptor neurons (ORNs): chemosensory[53] neurons in **sensilla** on arthropod appendages, especially the antenna, palp, or proboscis (Chapter 2). Perception begins when chemical vapor activates **odorant receptors** (ORs), **gustatory receptors** (GRs), and/or **ionotropic receptors** (IRs) located on the dendritic surface of ORNs that recognize biologically meaningful chemical ligands, governing their sensitivity and specificity, regulating innate and learned olfactory behaviors including attraction and repellency.[110,111] The expression of ORs follows the general rule of one OR to one ORN. Rather than binding specific ligands, ORs may display an affinity for a range of odor molecules and, conversely, a single odorant molecule may bind to a number of olfactory receptors with varying affinities, with some such as pheromone receptors showing high affinities (specificities). Specificity of each OR and thus the ORN is governed by concentration of the odorant to which it is exposed (Chapter 2). Insect ORs are atypical seven-transmembrane domain proteins (also known as G protein coupled receptors) that form ligand-gated ion channels by assembling a ligand-selective subunit with the **olfactory receptor coreceptor** (**Orco**).[104,145]

OR: acronym for odorant receptor.

Or: acronym for **odorant receptor gene**.

Orco: acronym for olfactory **receptor** coreceptor.

Organic: strictly, chemical compounds derived from plants or animals, plus other carbon-based materials. Essential oils from plants (Chapter 9) include many useful organic repellents. In the terminology of modern farming and horticulture, so-called organic vegetables and other agricultural produce are defined as those grown and marketed without the application of **synthetic** pesticides and other potential pollutants.[85,86]

ORN: acronym for olfactory receptor neuron; also called olfactory sensory neuron (OSN).

orthokinesis (*n*): change in speed of movement (velocity) of an organism, depending on the intensity of a stimulus.

OSN: olfactory sensory neuron, see **olfactory receptor neuron** (ORN).

palp (*eng.*)**, palpus** (Latin) (*sing.*)**, palpi** (*pl.*): the maxillary palp is a sensory[146] appendage on each of the paired maxillae (anterior to paired mandibles) under the head of an **arthropod**.

personal protective measures[112] **(PPMs)**: protective measures against biting arthropods, such as the personal use of repellents, bednets, and clothing.

pesticides: chemicals for killing pests, classified by the EPA as follows: algicides, antifouling agents, antimicrobials, biocides, biopesticides, defoliants, desiccants, disinfectants and sanitizers, fungicides, fumigants, herbicides, **insect** growth regulators, **insecticides**,

acaricides (including miticides), molluscicides, nematicides, ovicides, pheromones, plant growth regulators, rodenticides, and **repellents** (www.epa.gov/pesticides/about/types.htm).

pH: number expressing degrees of acidity (pH < 7) and alkalinity (pH > 7) in solutions; pH 7 is neutral. Mathematically, pH is the logarithm to base 10 of the reciprocal of hydrogen ion concentration; it is usually measured by comparison with a standard solution of potassium hydrogen phthalate, with a pH of 4 at 15°C.

phagomone: chemical that affects feeding behavior, negatively or positively, by any mode of action.

phagostimulant: something that elicits feeding.

phase: the physical state of material: vapor, liquid, or solid.

pheromone: a chemical compound, emitted by an organism, that influences the behavior and development of other members of the same species.

phlebotomine sandflies: a subfamily of stealthy bloodsucking flies (**Diptera**: Psychodidae: Phlebotominae) with approximately 700 species described, the main genera of importance being *Lutzomyia* in the New World and *Phlebotomus* in the Old World.

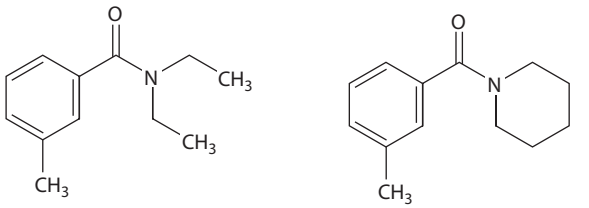
phytophagous (*adj.*)**, phytophagy** (*n*): plant eating (Greek: *phytos*), (*cf.* **hematophagous**).

phytopomorphize (*vb*): from the perspective of plants (*cf.* **anthropomorphize**).[32]

phytotoxicity: pathological effect on plant vegetation.

picaridin (KBR 3023): insect repellent developed and commercialized as Bayrepel®; approved by the **WHOPES**[66] and specifications issued in 2006 under the proposed ISO name icaridin.

piperamides and **piperidine alkaloids**: a series of compounds and analogs that includes many useful repellents, some being also insecticidal, e.g., **deet**, SS220, and pipernonaline.[113–116] The amides have a carbonyl (C=O) group linked to a nitrogen, N–(C=O), whereas the nitrogen's other two bonds are linked with hydrogens or other groups, e.g., **deet** (Figure 1.1). When the nitrogen joins a saturated heterocyclic ring with five carbons, the compound constitutes a piperidine—the chemical name derived from plants of the pepper family (Piperaceae) that contain many such natural compounds, sometimes used as repellents (Chapter 9). Natural piperidine (CAS number 110-89-4) is the noxious ingredient of the poison hemlock (*Conium maculatum*) in the carrot family Apiaceae. Among more than 200 such compounds identified in *Piper*,[117] the relatively simple amides provide much of the hot, pungent, and spice taste as well as the biological activity in many **species**.[112] The piperamides commonly found in the genus *Piper* are bifunctional; an isobutyl amide functionality is combined with a methylenedioxyphenyl (MDP) moiety, as seen in the piperine of *Piper nigrum* fruit and 4,5-dihydropiperlonguminine in foliage of the Central American *Piper tuberculatum*. The most active piperamide discovered to date is pipercide, which is approximately 100-fold more active than piperine.[118–120] The piperamides are also unusual because of their dual biological activities: the amide functionality is neurotoxic, and the MDP group is an inhibitor of cytochrome P450 enzymes. Scott et al.[121] demonstrated that



N,N-diethyl-3-methylbenzamide 1-(3-methylbenzoyl)piperidine

Figure 1.1 Deet (on the left) has a benzene ring linked by a carbonyl group (C=O) to an amide (piperamide) with two CH_3 (methyl) groups; the piperidine analog (on the right) has a saturated carbon ring that includes the nitrogen from the amide.

combinations of piperamides in binary, tertiary, and quarternary mixtures had successively higher toxicities at equimolar concentrations. This combination of useful traits suggests that *Piper* extracts may be good candidate pesticides with a rich range of insecticidal and repellent properties.

placebo (used as negative **control** for **bioassay**): an inert substance or treatment dosage that appears to be same as the active treatment.

p-menthane-3,8-diol (PMD): occurs naturally in the leaves of Australian lemon-scented gum tree (*Corymbia citriodora*), commonly called lemon eucalyptus.[122] This monoterpene, structurally similar to menthol (CAS number 42822-86-6), remains as a spent product after distillation of essential oils from the leaves and twigs of *Corymbia citriodora*. Whereas natural PMD-based repellents have long been popular in China and elsewhere,[123] and registered in Europe for over a decade, **synthetic** PMD is used as the active ingredient for some of the repellents marketed as lemon eucalyptus in the United States. As described in Chapters 12 and 14, PMD exerts repellency of the highest order against a wide range of hematophagous **arthropods**. Formulations registered in the United States include liquids that are sprayed on skin or clothing, or lotions that are rubbed on skin. Not yet submitted for WHOPES evaluation.

poison: any **toxic** substance that upsets normal physiological and biochemical mechanisms in a living organism, causing pathology and leading to death if the dosage is sufficient. Paracelsus in the sixteenth century was the first to recognize that "the dose makes the poison" as everything can be **toxic**, including **repellents**.[124]

PPM: acronym for personal protective measure[112] against biting arthropods, such as the use of topical repellents and clothing (*cf.* ppm, expressing dilution in terms of parts per million).

probe, probing[51] (*vb.*): the act of penetrating skin by the mouthparts of an insect or other arthropod without ingestion of blood. *cf.* bite

proboscis (*sing.*), **proboscides** or **proboscises** (*pl.*) (*n*): mouthparts of adult **hematophagous** arthropods, forming an anterior tubular prolongation on the head. The proboscis of adult mosquitoes and other biting flies (**Diptera**) consists of labium (upper sheath), labrum (lower sheath), paired mandibular stylets, paired maxillary stylets, and hypopharynx with salivary duct[125] bundled into a strong rostrum for biting, with a lumen canal for the fluid being ingested.

public health pesticides (http://ir4.rutgers.edu/publichealth.html): comprise a wide range of repellents, insecticides (adulticides and larvicides), insect growth regulators, rodenticides, lures, and their applications for controlling pests and vectors of public health importance, and personal protection from them.

pyrethrins: oily esters extracted from cultivated pyrethrum flowers, *Chrysanthemum cinerariaefolium* Vis., syn. *Tanacetum cinerariifolium* (Trevir); also found in pyrethrum daisies: *Chrysanthemum roseum* Web. & Mohr., syn. *Chrysanthemum coccineum* Willd (Asteraceae). The crude pyrethrin extract contains three esters of chrysanthemic acid (chrysanthemates: pyrethrin I, cinerin I, and jasmolin I) plus three esters of pyrethrin acid (pyrethrates: pyrethrin II, cinerin II, and jasmolin II), combined ratio 71:21:7, generally known as pyrethrins. Being lipophilic but having low aqueous solubility, pyrethrins are readily absorbed via arthropod cuticle but not via the skin of vertebrates. Pyrethrins are very potent insecticidal **knockdown** agents, causing **excitorepellency** at sublethal doses, due to the disruption of sodium channel gating in myelinated nerves. Commercially, 25%–50% pyrethrin concentrates are very stable in darkness at ambient temperatures, but they degrade rapidly in sunlight (DT_{50} of 10–12 minutes).

pyrethroids: numerous **synthetic** organic compounds, mostly based on the chrysanthemate moiety of pyrethrum, having analogous neurotoxic modes of action causing rapid **knockdown** and **insecticidal** effects. Discovery and development of synthetic pyrethroids, during the

1960s and 1970s, accomplished several goals: more economical and consistent production than with natural pyrethrins; photostable products with residual efficacy but limited bioaccumulation. After early progress with allethrins (transient space sprays and vaporizers), the first truly stable pyrethroids were fenvalerate and permethrin; their relative safety and potency greatly surpassed those of other classes of insecticides. Wide variations in potency occur between *cis* and *trans* isomers, and among enantiomers of pyrethroids, allowing much diversity in pyrethroid products, providing manufacturers and users with choices between knockdown versus insecticidal potency and degrees of residual stability. With the commercialization of hundreds of pyrethroids, this class of compounds has come to dominate the insecticide industry during recent decades. Permethrin remains one of the favorites for its versatility as an **insecticide** with **repellent** and **deterrent** properties (Chapters 6, 7, 17, and 18). The other pyrethroids mentioned in this book include allethrins, α-cypermethrin, β-cyfluthrin, deltamethrin, esbiothrin, λ-cyhalothrin, metofluthrin, prallethrin, tetramethrin, and transfluthrin.

questing[51]: behavior of **ticks** or **chiggers** actively seeking a host.

REACH: Registration, Evaluation, Authorisation and Restriction of Chemical substances, the European Community Regulation (EC 1907/2006) on chemicals and their safe use (http://ec.europa.eu/environment/chemicals/reach/reach_intro.htm) coordinated by the European Chemicals Agency in Helsinki, Finland (http://echa.europa.eu/regulations/reach/legislation).

receptor: sensory system protein, cell, organ, or other structure; *ref.* **OR**, **Orco**, **ORN** (Chapters 2 and 3).

refractoriness, refractory vector: genetic physiological inability of a potential vector to support and transmit particular agents of vector-borne disease (*cf.* **vector competence**).

repellency duration[20,51,127]: measured by the complete protection time (CPT), being the amount of time (minutes, hours, or days) that a compound will fully protect against bites of mosquitoes, etc. The end point is normally measured as the "time to first bite," although the "time to second bite" may be used to provide the "time to the first confirmed bite." For minimizing variance in time until the first or second bite as the end point, despite CPT being an easy metric to compare repellent efficacies, the end point is usually a threshold number of bites.

repellant, repellent: something that causes insects to make oriented movements away from its source.[7] Associated terms: to repel (*vb*); repellency (repellancy) (*n*), the quality of repelling; repeller (*n*), device for repelling (invalid for electronic[1] so-called mosquito repellers[126] or buzzers); repulsion (*n*), the act of repelling or the state of being repelled; and repulsive (*adj.*), serving to repel. EPA test guidelines[51] define a repellent as "a product intended to disrupt the host-seeking behavior of insects or other arthropods, driving or keeping them away from treated human skin." The term repellent has received such general usage as a formulated product or as a chemical with a specific behavioral effect that it has lost much of its technical meaning. The editors of this book advocate that the term repellent should be restricted to the designation of products intended to reduce the rate of biting from hematophagous arthropods (French: *insectifuges corporels*). In this way, the technical literature will tend to use more precise terms that describe the effects of chemicals on specific behaviors. The introduction of the term **phagomone** is, in part, an attempt to facilitate this transition by providing the technical literature with an alternative to the term repellent used generally.

repellent testing criteria: see **dose**[50] and **repellency duration**.[20]

resistant, resistance: defined by the WHO (1957)[128] as "the development of an ability in a strain of some organism to tolerate doses of a toxicant that would prove lethal to a majority of individuals in a normal (susceptible) population of the same species," various types of insecticide resistance are well known in many species of flies, mosquitoes, and other vectors and

pests of public health importance.[129] For an increasing number of species, diagnostic and discriminating dosages have been determined[130] for distinguishing between susceptible and resistant individuals. Selection for resistance against **repellents** might be expected, due to their ubiquitous usage and environmental persistence[131,132] of **deet**. Because no effort has been made to monitor the sensitivity of wild populations of the many arthropod species that repellents are used against, the possibilities of behavioral or physiological resistance to repellents remain unexplored. However, studies with laboratory strains of **mosquitoes** and *Drosophila*[135] demonstrate genetic selection of for insensitivity[153] to deet, as well as reduced repellency following previous exposure,[154] indicating the potential for resistance to deet and other **repellents**. Previous reports of **tolerance**[133,134] and resistance[155] to deet involved comparisons between different species, not studies of inheritance or conditioning, although some intriguing contrasts were detected between strains of Anopheles albimanus[155] in their responses to deet and its resolved isomer SS220.

risk: probability of hazard; toxicity × exposure; risk assessment in the context of human health, estimating the probability of adverse effects resulting from defined exposure to a known chemical **hazard**.[87] See Chapters 16 and 17 for repellents; minimum risk; reduced risk.

Rutgers 612: the original proprietary name for ethyl hexanediol (CAS number 94-96-2) when used as a repellent product; withdrawn in 1991 for toxicological reasons.

Rutgers 6-2-2: a repellent mixture consisting of six parts dimethyl phthalate, two parts Rutgers 612, and two parts indalone, optimized for military[4,12] use during World War II as M-250.

sandflies: *ref.* **biting midges** (Ceratopogonidae) and **phlebotomine sandflies** (Phlebotominae).

semiochemicals: chemicals involved in communication among organisms.[136]

sensilla (*pl.*)**, sensillum** (*sing.*) (*n*): numerous **cuticular** sensilla of several types are present on the **antennae**, **palpi,** and **proboscis** of **hematophagous arthropods**. For mosquitoes and other biting **Diptera**, those involved in olfaction include sensilla basiconica, sensilla coeloconica, and sensilla trichodea.[138,149,150] Ticks have olfactory sensilla clustered in and around Haller's organ on the foreleg tarsus.[137]

SI units: International System of units, based on metric values (http://physics.nist.gov/cuu/Units/index.html).

smell (*n or vb*): to detect or sense an odor (*amer.*), odour (*eng.*).

soluble: ability to dissolve in a given solvent, such as acetone, alcohol, and water.

solute: that which dissolves.

solution: solvent plus solute.

solvent: liquid in which solute dissolves to form solution.

space or spatial repellent (*n*)**, space or spatial repellency** (*vb*): repellent vapor, effective at a distance[10,138,147] for protecting people against mosquitoes and other biting flies in a defined area or space, e.g., room or backyard. **Area repellent** (Chapter 12) is an easier term with the same meaning. The most commonly employed space repellents are certain volatile **pyrethroids**: allethrins, metofluthrin, transfluthrin, and some **essential oils** (e.g., geraniol, and linalool) when vaporized from **emanators** and **dispensers** (so-called **CAFIKs**, LEDs, and heated mats), paper fans and strips, and burning coils and candles.[64,139,140] Spatial repellency depends on behavioral reactions induced by airborne chemicals that cause arthropods to move away.[63] Resulting protection against arthropods may be through multiple modes of action:

1. arthropods do not enter the treated space **(deterrence)**.
2. arthropods exert an oriented avoidance reaction **(taxis)**.
3. insects exert increased undirected movement **(orthokinesis)**.
4. repellents inhibit insect responses to an attractive target (inhibition) through **agonism** or **antagonism** of insect **odorant receptors (ORs)** or **coreceptors (Orcos)**.

species (*sing.* and *pl.*): abbreviations are as follows: sp., one species; spp., multiple species; ssp., subspecies. Each biological species comprises "members of populations that actually or potentially interbreed in nature." Normally, species do not interbreed, because they have premating (behavioral) and postmating (genetic) reproductive barriers. In biosystematics and nomenclature, groups of closely related species are classified as genera (*pl.*). Formal names for each **genus** (*sing.*) and species are published in accordance with the International Code of Zoological Nomenclature (http://iczn.org/code) following the Linnaean system of binomial nomenclature. Examples of different mosquito species names in the same genus are *Anopheles funestus* Giles 1900 and *Anopheles gambiae* Giles 1902. Both were described by the same author George Giles, and they were represented by the author's surname and the year of publication of each species name. Related genera are classified into families (e.g., Culicidae for mosquitoes in genera *Aedes, Anopheles, Culex, Mansonia*, etc.), and related families are classified into orders, e.g., **Diptera** for two-winged flies, including the families Ceratopoginidae (biting midges), Culicidae **(mosquitoes)**, Muscidae (face flies, houseflies, etc.), Simuliidae (blackflies), and Tabanidae (deerflies, horseflies, etc.) and >100 other genera, with about 150,000 species of named flies (plus many more species that remain undescribed). Several hundred species of insects are likely to bite humans, justifying the use of repellents for personal protection.

specifications: standard descriptions of products for quality control purposes. For **repellents** and other **pesticides**, international specifications are prepared by the Food and Agriculture Organization (FAO) and/or the World Health Organization (**WHO**), and then adopted by the FAO/WHO Joint Meeting on Pesticide Specifications (http://www.who.int/whopes/quality/en/), in conjunction with **CIPAC** analytical methods. Joint FAO/WHO specifications are issued by the WHOPES, available only in electronic format from http://www.who.int/whopes/quality/en, providing a qualitative basis for production and procurement.

spreader: a chemical that increases the area that a certain volume of liquid will cover.

sticker: something increasing adherence; **formulation ingredient** to enhance adherence of the **active ingredient**.

stimulants: substances that cause insects to begin moving, copulating, feeding, or laying eggs[6]; hence, qualified terms such as **locomotor stimulant,** mating stimulant, and oviposition stimulant. The term feeding stimulant is synonymous with **phagostimulant**.[141]

substrate: for purposes of repellents and other pesticides, the substrate is a treated surface (*cf.* biochemical substrate, molecule acted upon by an enzyme; bioecological substrate, environment in which an organism lives).

surfactant: chemical agent that increases the emulsifying, dispersing, spreading, and/or wetting properties of another chemical when contacting a surface.

suspension: finely divided solid particles mixed in a liquid in which they are not soluble.

synergist: a substance that, when combined with another substance, gives an effect that is greater than the sum of their individual effects, e.g., carbon dioxide plus lactic acid for synergism[38] of mosquito attraction.

synomone: mutually beneficial signal chemical released by members of one species that affects the behavior of another species and benefits the individuals of both the species.

synthetic: chemical compounds made by human-directed processes, as opposed to those of **natural** origin; the same material may be produced naturally or synthetically (e.g., PMD, Chapters 9 and 19. Since the 1940s, most commercial repellents are synthetic compounds. Synthetic **pyrethroids** are important insecticides and irritant repellents, usually including a chrysanthemic moiety homologous to natural **pyrethrins**.

taxis: directional response to stimulus. Movement toward the source being positive taxis; movement away from the source being negative taxis (*cf.* **kinesis**). chemotaxis (*n*), chemotactic (*adj.*): movement responding to chemical (**attractant** or **repellent**).

ticks: acarine suborder Ixodida, with two important families: hard ticks (Ixodidae) and soft ticks (Argasidae), all approximately 650 species depending on repeated blood meals for growth and reproduction.

technical-grade chemical: unpurified after synthesis, containing small amounts of impurities (other chemical precursors and by-products), economical product for commercial formulation. For instance, technical-grade **deet** is usually 95% *N*,*N*-diethyl-*meta*-toluamide, with *ortho* and *para* isomers of **deet** being the other 5% as less potent repellent ingredients.

time: to first bite; to first confirmed bite; to second bite: repellent testing criteria, *ref.* **dosage** and **repellency duration**.

tolerance: having low susceptibility to particular pesticides due to high fitness of the individual or population; usually attributable to the presence of some robust or resistant individuals from which a more obviously **resistant** population can be selected (due to increased frequency of resistant genotypes when successive generations are subjected to Darwinian selection). In many countries, regulatory systems set *pesticide tolerances* as maximum permissible levels of residues in foodstuffs, and so on (established by the **EPA** in the United States and by the **ECB** in the EU). Tolerance has special meaning for quality control purposes, whereby the permissible range of variation is defined in product **specifications** with respect to the **active ingredient**, e.g., mean $\pm$ 10%, possibly expressed as variance. Using this mathematical concept, Rutledge[133,134] assessed repellent tolerances of mosquito populations to compare ranges of responses and resistance potential. Generally, for pesticides tolerance is recognized when the LC_{50} of a population rises up to five times greater than normal for a standard susceptible strain of the same species; higher ratios (dose–efficacy comparisons between populations of the same species) indicate **resistance**.

topical repellent: a **repellent** applied to the skin for preventing bites from **hematophagous** arthropods.

toxics: based on the Greek word *toxikon* for arrow poison; toxicology is the study of poisons, biologically harmful substances and their effects. Dose-dependent criteria allow any material to be toxic, serving as a **toxicant** or **toxin** for sensitive tissues or organisms, although this term is normally applied to hazardous pathogens, pesticides,[87] and other chemicals. **Toxicity** of pesticides is commonly measured (for each species) in terms of lethal concentrations or dosages at the 50% level (LC_{50} or LD_{50}) and the 99% level (LC_{99} or LD_{99}) for comparative purposes when dealing with target insects and nontarget species. The Toxics Release Inventory is a publicly available EPA database (http://www.epa.gov/tri/) that contains information on toxic chemical releases and other waste management activities reported annually by industry and U.S. federal facilities. For chemical safety purposes, in setting tolerances (as mentioned earlier), toxicologists determine the *no observed adverse effect level* for laboratory animals. Mammalian toxicity values, required by regulatory authorities (such as the USEPA, Chapter 17) for assessing pesticides for regulatory approval, are based on effects of short-term (acute), long-term (chronic), and intermediate (subchronic) periods of exposure, as well as effects on development and reproduction, including mutagenicity and carcinogenicity, to establish dose–response relationships. For instance, acute tests (the so-called six-pack) comprise oral, dermal and inhalation lethal dosages (LDs); neurotoxicity; eye irritation; dermal irritation; and sensitization (www.epa.gov/oppfead1/trac/a-toxreq.htm). The human equivalency potency factor (Q) is usually based on the oral exposure route; it is designated as Q^* when considered carcinogenic (www.epa.gov/pesticides/carlist). The so-called reference dose (RfD) is the average daily oral exposure that is estimated to be unlikely to cause harmful effects during a lifetime. RfDs are generally used by the EPA for health effects that are thought to have a low threshold (dose limit) for producing effects. The International Programme on Chemical Safety[142] emphasizes the acceptable daily intake[142] for each chemical, aggregated from all sources

of exposure, whereas the USEPA increasingly considers cumulative risk (www.epa.gov/oppsrrd1/cumulative/) from exposure to groups of pesticides with an equivalent mode of action (e.g., organophosphates). Whereas the mode of action of insect repellents is not well understood (Chapter 11), the toxicology of repellent compounds is not difficult to assess by standard methods.

U.K.: United Kingdom of Great Britain and Northern Ireland, one of the 28 member states of the European Union, and therefore subject to the **Biocidal Products Directive** for pesticides, under the **REACH** regulations for chemicals.

USDA (United States Department of Agriculture): it has a variety of agencies, offices, and services, notably the Agricultural Research Service (ARS) with long-term research on insect attractants and repellents.

USEPA, OPP (United States Environmental Protection Agency, Office of Pesticide Programs) (http://www.epa.gov/pesticides/): comprises several operating divisions, currently named as follows: Antimicrobials, Biological and Economic Analysis, Biopesticides and Pollution Prevention, Environmental Fate and Effects, Field and External Affairs, Health Effects Division, Information Technology and Resources Management, Registration, Special Review, and Reregistration. Collectively, they are responsible for pesticide regulatory management in the United States.

vapor pressure: the property causing a chemical to evaporate, defined as the pressure of the vapor in equilibrium with the liquid or solid state; measured in joules, **SI** units of energy (http://physics.nist.gov/cuu/Units/index.html).

vector: carrier of infection. Vector-borne pathogens cause disease, e.g., *Plasmodium* causes malaria, which is transmitted by the vector *Anopheles* mosquito.

vector-borne diseases: infectious diseases for which the causal agent must be transmitted by competent vectors, such as the mosquito *Aedes aegypti* as the main vector of dengue virus, various *Anopheles* spp. as the vectors of malaria *Plasmodium* spp., and certain tick *Ixodes* spp. as the vectors of *Borrelia burgdorferi* causing Lyme disease. Hence the needs for vector control and **personal protective measures** including the use of **repellents**.

vector competence: ability of a vector to support and transmit particular agents of vector-borne diseases, e.g., *Brugia malayi* filariasis, dengue virus, and *Plasmodium vivax* malaria (*cf.* **refractoriness)**. Repellents do not affect vector competence, although there is mounting evidence that infective vectors may be less sensitive to repellents in some circumstances (Chapter 20).

vector potential: local presence of competent vectors likely to transmit, whether or not the subject agent of vector-borne disease is present; *ref.* **vector competence**.

vectorial capacity: for vector-borne diseases, the dynamic relationship between populations of the host and the pathogen via competent vectors[148]. For human malarias[151]: $C = ma^2p^n/-\log_e p$, where C = vectorial capacity, m = density of vectors in relation to humans, a = number of human blood meals per vector per day, p = daily survival probability of vectors (measured in days), n = incubation period in the vector (measured in days), and $-\log_e$ is the natural log value (of p). This formula expresses the capacity of the vector population to transmit the infection, based on the potential number of secondary inoculations originating per day from an infective person. Values in the formula differ between vector species for intrinsic and environmental reasons. Where multiple vector species coexist, C is the sum of the vectorial capacities of all those vector species. Repellents affect human–vector contact, mainly reducing ma^2 and thus impacting vectorial capacity.

viscosity: the property of liquids to resist flow due to forces acting between the molecules. The **SI** physical unit of dynamic viscosity (Greek symbol: μ) is the pascal second (Pa·s), identical to 1 Ns/m^2 or 1 kg/ms.

volatile: substance with high **vapor pressure**, readily evaporating to vapor phase; plant volatile blends attract phytophagous host specifics[111] but are generally repulsive to prevent herbivory; some (e.g., **PMD** and **undecanone**) are useful repellents against **hematophagous** arthropods.

volatility: rate of evaporation of material from liquid or solid phase.

wetting agent: a chemical that increases the liquid contact of dry material.

WHO and **WHOPES**: the World Health Organization and the WHO Pesticides Evaluation Scheme, responsible for assessments, specifications, and recommendations for pesticides (including repellents) used for public health pest and vector control[143] on behalf of member states of the United Nations (http://www.who.int/whopes/en/).

xenobiotic: any other material from outside the subject organism.

zoophagy, zoophily: tendency of **hematophagous** insects to bite or prefer hosts other than humans (*cf.* **anthropophagy, anthropophily**).

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CHAPTER 2

Neuromolecular Basis of Repellent Action

Joseph C. Dickens and Jonathan D. Bohbot

CONTENTS

INTRODUCTION

Physical contact is not required for insect repellents to affect mosquito behavior; *N*,*N*-diethyl-3-methylbenzamide (deet) not only interferes with the detection of host and oviposition sites,[1] suggesting the involvement of the olfactory pathway, but also deters feeding,[2] perhaps indicating the involvement of the gustatory sense.[3] However, the broad activity of these compounds and their required quantities to repel arthropods are puzzling characteristics. More confounding is the fact that deet and other insect repellents do not prevent long-range attraction but rather perturb mosquito behavior at relatively close ranges.[4]

Our understanding of the neuromolecular mode of action of repellents has largely followed the development of methods. In 1962, Jurgen Boeckh[5] reported the first single-cell recordings from an insect olfactory receptor neuron (ORN) in the *Necrophorous* carrion beetle. A few years later, in 1967 Lacher[6] made the initial electrophysiological recordings from ORNs in mosquitoes. He showed that volatile stimuli either excited or depressed the activity of individual ORNs. Davis and Sokolove[7] showed in 1976 that responses of ORNs to an attractant, lactic acid, were inhibited by deet. Although techniques for electrophysiological recordings from gustatory receptor neurons (GRNs) in insects were first described by Hodgson et al. in 1955,[8] only recently have recordings been made from GRNs in mosquitoes in response to insect repellents.[3]

With the discovery and characterization of odorant receptors (ORs) in insects,[9–11] the pioneering in vivo electrophysiological studies were followed by functional studies of insect ORs expressed ex vivo in heterologous expression systems such as human embryonic kidney cells,[12] the *Drosophila*

empty neuron system,[13] and frog eggs.[14] These heterologous expression systems have allowed for the determination of the role of ORs in the specificity of ORNs and have provided platforms for high-throughput screening of chemicals resulting in the discovery of an OR allosteric agonist.[15] Although molecular studies have implied certain gustatory receptors (GRs) to be involved in detection of deet in the vinegar fly *Drosophila melanogaster*,[16] detailed pharmacological investigations of the effects of insect repellents on specific GRs remain to be conducted.

Following a description of the molecular components involved in early chemosensory events in insects, this chapter presents the current knowledge of the mode of action of repellents on olfactory and gustatory processes with a special focus on studies involving mosquitoes. This chapter also presents a theoretical model of peripheral and central neural responses to blends of odorants and insect repellents that lead to the disruption of normal behavior in insects.

CHEMICAL SIGNALING IN INSECTS

The chemical sensing system of insects comprises both olfactory and gustatory sensilla. Olfactory sensilla are generally located on the antennae and maxillary palps (Figure 2.1a) of mosquitoes, but a few olfactory sensilla have been reported on the proboscis.[17] The ORNs, generally express only one ORN, send their axons to a region of the brain called the antennal lobe where glomeruli receive input from ORNs of similar specificity[18,19] (Figure 2.1b). Olfactory sensilla are multiporous and house the dendrites of several ORNs (Figure 2.1c). The aqueous sensillum lymph surrounding the dendrites comprises numerous ions as well as several proteins involved in olfactory processes. Odorant-binding proteins (OBPs) show a degree of specificity for odorants and are thought to facilitate their transport to ORNs located in the outer membrane of dendrites.[11] The ORNs paired with an obligatory olfactory receptor coreceptor Orco[20] form ligand-gated ion channels[21,22] and, to a large degree, determine the specificity of the ORN expressing them.[23,24] Sensory neuron membrane protein 1[25] may serve as a docking point for pheromone-binding proteins (PBPs), transporting pheromone molecules to an OR.[26] Finally, inactivation of odorants in the sensillum lymph may be carried out by a variety of enzymes called odorant-degrading enzymes (ODEs).[27–29]

Gustatory sensilla have a terminal pore that allows access to nonvolatile chemicals (Figure 2.1d). Associated with each gustatory sensillum are dendritic processes from GRNs.[30] The tips of these dendrites extend to the terminal opening of the sensillum where GRs interact with chemicals passing into the sensillum. In contrast to ORNs, GRNs may express several GRs. Whether GRs are G protein–coupled receptors or ligand-gated ion channels have not yet been determined (Figure 2.1d). However, single GRNs generally serve as labeled lines for coding different tastes such as salt, sugar, and bitter or aversive compounds. Axons from GRNs expressing a specific GR protein project to distinct regions of the subesophageal ganglion[31] (Figure 2.1b).

INSECT REPELLENTS AS OLFACTORY RECEPTOR AGONISTS

Insect ORs were first identified in the vinegar fly *D. melanogaster* in 1999.[9–11] Three years later, the release of the malaria vector, *Anopheles gambiae*, genome[32] led to the identification of complete chemoreceptor gene families including ORs.[33] The first OR ligands for *A. gambiae* ORs (AgORs) were identified in 2004,[34] and several cell-based assays using ORs were established shortly thereafter, paving the way for pharmacological studies of mosquito ORs.[35,36]

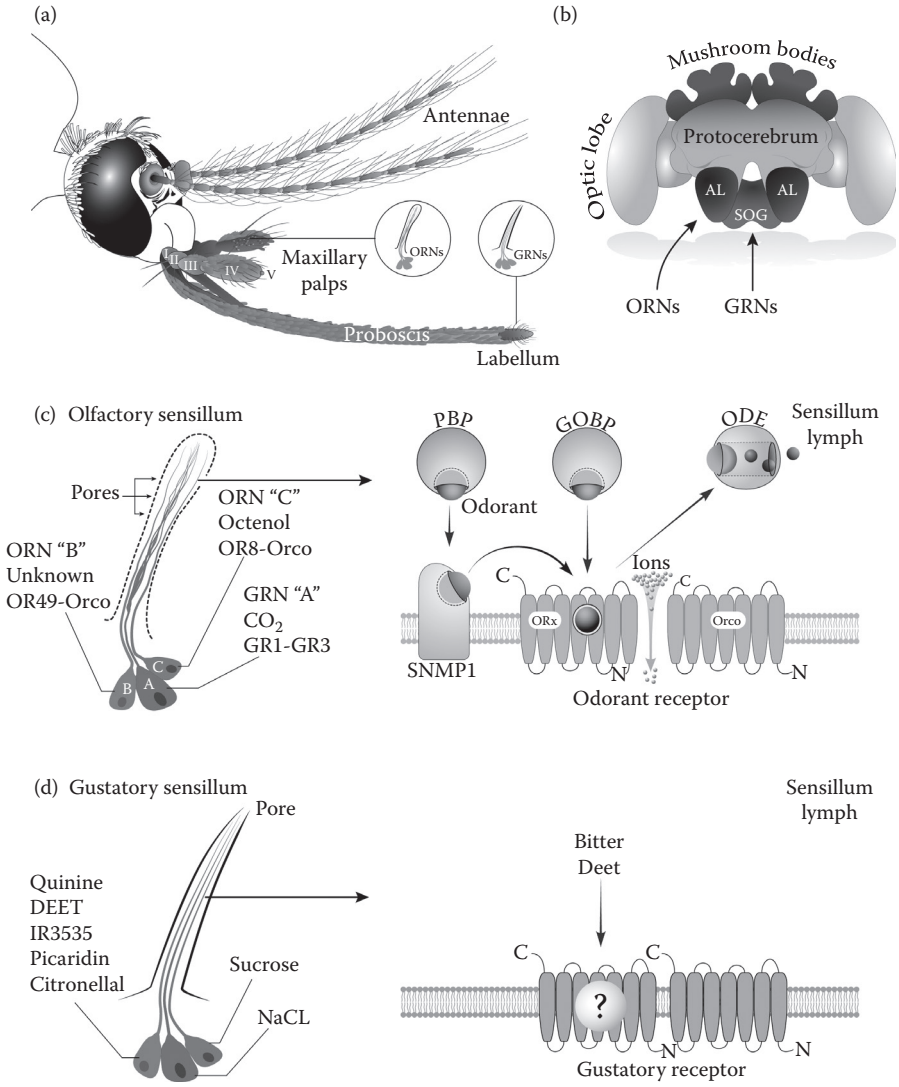


Figure 2.1 **(See color insert.)** Chemical sensing in the female *Aedes aegypti* mosquito. (a) The peripheral olfactory system is distributed onto three types of appendages on the head of mosquitoes: the antennae, the maxillary palps, and at the extremity of the proboscis (labellum). (b) Chemosensory information detected by olfactory receptor neurons (ORNs) and gustatory receptor neurons (GRNs) is sent to the antennal lobe (AL) and subesophageal ganglion (SOG) in the brain. (c) Basiconic sensilla are located on the surface of the fourth segment (IV) of the maxillary palp. Multiple pores in the cuticle allow odorants to interact with ORNs. ORN "A" responds to CO_2 with the largest amplitude action potential, via the activation of at least two gustatory receptors (GR1 & GR3). ORN "B" responds to an unknown odorant with an intermediate size action potential. 1-Octen-3-ol elicits the smallest action potential from ORN "C". In *Aedes aegypti*, ORNs "B" and "C" are thought to express OR49-Orco and OR8-Orco assemblages, respectively. Accessory proteins, both soluble and membrane bound, are thought to participate in the activation of ORs. Several possible models include pheromone-binding proteins (PBPs) delivering the odorant to sensory neuron membrane protein 1 (SNMP1), which in turn offloads the odorant to the receptor. An alternative possibility is that a general odorant-binding protein (GOBP) directly transfers the odorant to the receptor. (d) Gustatory sensilla are located on the labellum and legs[76] of mosquitoes, and perhaps wing margins as shown in *Drosophila melanogaster*.[30] Gustatory sensilla located on the labellum process at least three types of compounds: salt, sweet, and bitter. The topology, stoichiometry, and potential molecular partners of GRs are not well understood.

Three independent and pioneering studies have proposed seemingly conflicting theories on the potential mode of action of deet on olfactory pathways. Deet alone activated AgOR40, a receptor expressed in larval antennae (Figure 2.2), when expressed in *Xenopus* oocytes, which suggested an agonist mode of action.[37] Deet and other repellents excited an ORN in a short trichoid sensillum on the antenna of the southern house mosquito, *Culex quinquefasciatus*,[38] as reported for the yellow fever mosquito, *Aedes aegypti*.[39] In another set of similar experiments, the activation of *D. melanogaster* OR47a and AgOR1, 2, and 8 by odorants was inhibited by deet, suggesting an inhibitory or antagonistic mechanism.[40] A third report showed that deet may directly interact with the mosquito attractant octenol (1-octen-3-ol), thereby reducing the amount of stimulus reaching the dendritic surface of the ORNs in *C. quinquefasciatus*.[38] However, this effect was refuted in a subsequent study that showed deet did not sequester octenol.[41] Pharmacological and physiological studies provide support for aspects of some of these theories.

Detailed pharmacological studies using *Xenopus* oocytes as an ex vivo heterologous expression system support the notion that deet and other insect repellents interfere with OR activation in a variety of ways. The mosquito OR2 and OR10 specifically recognize indole[42] and 3-methylindole (skatole),[43] respectively. OR8, one of the most studied and best understood mosquito receptors, discriminates between the two enantiomers of 1-octen-3-ol.[44] In *Anopheles gambiae*, OR8 is expressed in one of the three neurons within each basiconic sensillum on the surface of the maxillary palps.[45] The other two neurons respond to either CO_2 or an unknown compound. The "C" neuron, presumably expressing OR8, is also able to discriminate octenol enantiomers.[46,47] Moreover, OR8 activation by the racemic mixture parallels responses of the octenol-sensitive neuron (Figure 2.3). Although other proteins have been implicated in perireceptor events, such as OBPs, sensory neuron membrane proteins, and ODEs, ORs alone are sufficient to account for this high degree of ligand selectivity and sensitivity.

To test the theory that insect repellents act as OR agonists, several *Aedes aegypti* ORs (AaORs) including AaOR2, AaOR8, and AaOR10 were expressed with Orco and challenged with deet.[48] Deet alone activated AaOR2-Orco (Figure 2.2), but did not activate AaOR10-Orco.[49] Although deet and indole share some structural features, suggesting that both compounds interact with the same recognition site on the receptor, it is surprising that deet did not activate AaOR10-Orco. Based on a similar argument, it was expected that deet would not activate AaOR8-Orco, the octenol receptor. These results suggest that deet is interacting with the odorant-sensing subunit.[48] Conversely, 2-undecanone (2U) activated AaOR8-Orco, but did not activate AaOR2-Orco. The 2U is a broad-activity insect repellent[50,51] of natural origin produced by tomato plants[52] and has a 11-member carbon chain harboring a ketone function not unlike some octenol analogs, for example, 1-octen-3-one, that activate AaOR8-Orco, albeit at high concentrations (Figure 2.3a and b). The fact that several octenol analogs activate AaOR8 (Figure 2.3b) supports the idea that 2U and octenol interact with the same recognition site on AaOR8. The agonist effect of 2U was also observed in vivo on the octenol-sensitive neuron[46] (Figure 2.3a). Deet did not inhibit the response of the octenol neuron to octenol (data not shown), suggesting that this compound does not reach the receptor because of the factors present in the sensillum lymph. Recently, a high-resolution crystal structure of the *Anopheles gambiae* OBP1 (AgOBP1) revealed deet lodged within the protein-binding site.[53] Further binding experiments have confirmed deet-AgOBP1[54] and deet-AgOBP20[55] complexes. Murphy et al.[54] proposed that deet and other insect repellents disrupt the AgOBP1-indole-OBP4 complex, thus inhibiting odorant detection of the agonist. Single-cell recordings from *Drosophila* ORNs showed that deet modulated the response of several ORNs in the presence of odorant.[41] 1-(3-Cyclohexen-1-ylcarbonyl)-2-methylpiperidine (SS220) also activated AaOR2-Orco.[56] Finally, callicarpenal, a complex compound structurally quite different from octenol, selectively activated AaOR8-Orco, providing additional support for the existence of secondary recognition sites (allosteric sites) on this receptor.[56]

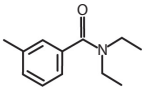
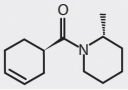
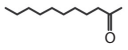
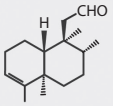
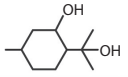
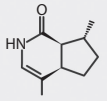
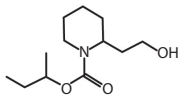
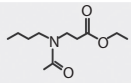
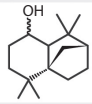
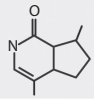
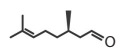
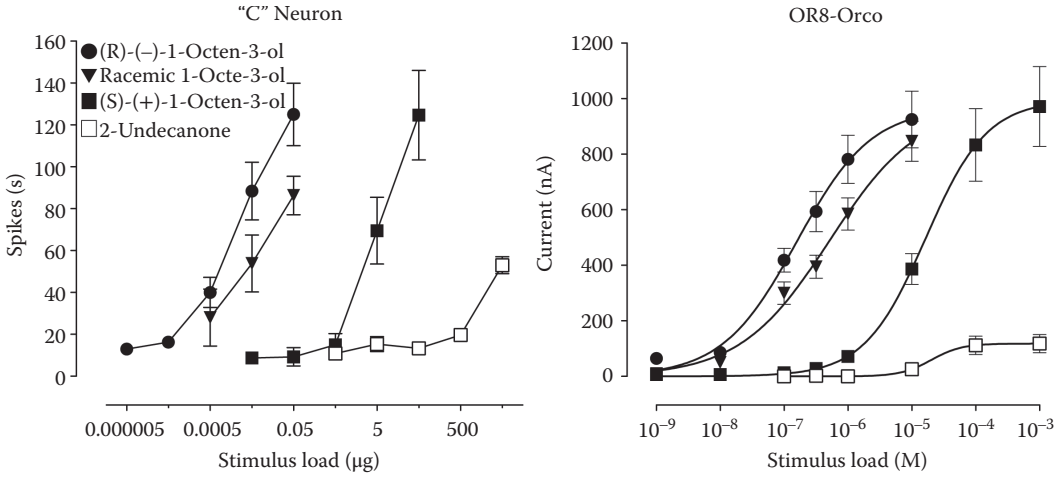
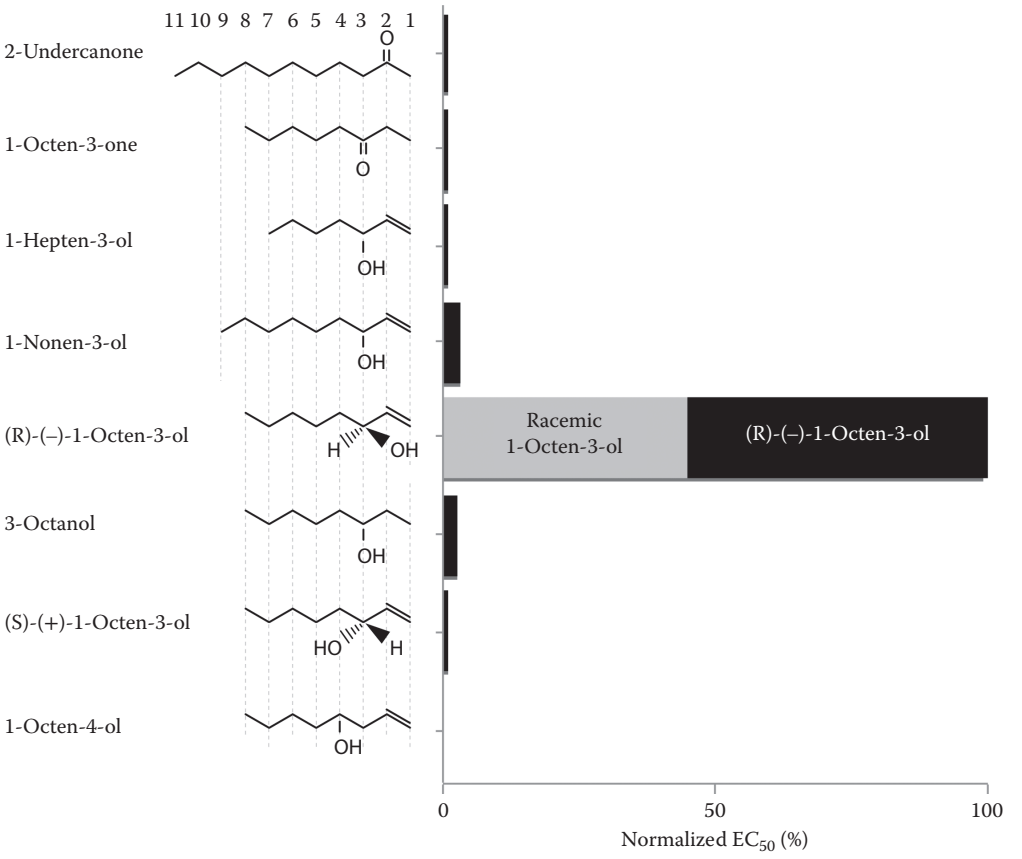
Insect repellent	Structure	Olfactory protein	Agoinst	Antagonist	Ligand	References
DEET *N,N*-diethyl-3-methylbenzamide		AgOR40-Orco	■			38
		AgOR1-Orco		■		41
		AgOR2-Orco		■		
		AgOR8-Orco		■		
		DmOR47A-Orco		■		41
		DmOR59B		■		42
		AaOR2-Orco	■	■		49, 50, 57
		AaOR8-Orco		■		
		AaOR10-Orco		■		
		AaGRx?	■			3
		AgOBP1 & 20			■	54, 55, 56
S220 1-[3-Cyclohexen-1-ylcarbonyl] -2-methylpiperidine		AaOR2-Orco	■	■		57
		AaOR8-Orco	■	■		
2U 2-Undecanone		AaOR2-Orco		■		49, 57
		AaOR8-Orco	■	■		
Callicarpenal [(1*S*,2*R*,4a*R*,8a*R*)-1,2,4*a*, 5-Tetramethyl-1,2,3,4,4*a*,7,8, 8*a*-octahydro-1-naphthalenyl] acetaldehyde		AaOR2-Orco		■		57
		AaOR8-Orco	■	■		
PMD *Para*-menthane-3,8-diol, 2-(2-hydroxypropan-2-yl)- 5-methylcyclohexanol		AaOR2-Orco		■		57
		AaOR8-Orco	■	■		
Nepetalactam (4*aS*,7*S*,7*aR*)-4,7-Dimethyl-2, 4a,5,6,7,7a-hexahydro- 1H-cyclopenta[c] pyridin-1- one		AaOR2-Orco	■	■		57
		AaOR8-Orco	■	■		
Picaridin 2-(2-Hydroxyethyl)-1- piperidine carboxylic acid 1-methylpropyl ester		AaOR2-Orco		■		49, 57
		AaOR8-Orco		■		49, 57
		AaGRx?	■			3
IR3535 3-[*N*-Butyl-*N*-acetyl]-amino- propionic acid ethyl ester		AaOR2-Orco		■		49, 50, 57
		AaOR8-Orco		■		
		AaOR10-Orco		■		
		AaGRx?	■			3
Isolongifolan-8-ol 2,2,8,8-Tetramethyl-octahydro-1*H*-2, 4*a*-methanonapthalene-10-ol		AaOR2-Orco		■		57
		AaOR8-Orco		■		
Nepetalactone (4*aS*,7*S*,7*aR*)-4, 7-Dimethyl- 5,6,7,7*a*-tetrahydrocyclopenta [c]pyran-1(4*aH*)-one		AaOR2-Orco		■		57
		AaOR8-Orco				
Citronellal (±)-3,7-Dimethyl-6-octenal		AgTRPA1	■			72
		DmOrco	?	?		72
		AaGRx?	■			3

Figure 2.2 Modulatory effects of insect repellents on olfactory proteins. Insect repellents largely exert their effects through the inhibition of odorant-activated *Aedes aegypti* (AaORx-Orco), *Anopheles gambiae* (AgORx-Orco) and *Drosophila melanogaster* (DmORx-Orco) odorant receptors. These compounds may also interact with an *Aedes aegypti* gustatory receptor (AaGRx) yet to be identified. Deet also forms complexes with *Anopheles gambiae* odorant-binding proteins (AgOBPs). Citronellal affects the function of an *Anopheles gambiae* transient receptor potential A1 (TRPA1) channel, a *D. melanogaster* Orco (DmOrco), and potentially an AaGRx.



(a)



(b)

Figure 2.3 Molecular and cellular bases of octenol detection. (a) The "C" neuron discriminates between the (*R*)-(–)- and (*S*)-(+)-enantiomers of 1-octen-3-ol. The OR8-Orco complex shows the same selectivity toward the two enantiomers of octenol. Notice the similarity of both systems toward the racemic mixture of 1-octen-3-ol and the insect repellent 2-undecanone. (b) The sensitivity of OR8-Orco toward each structural analog of (*R*)-(–)-1-octen-3-ol is characterized by EC_{50} values relative to the EC_{50} of (*R*)-(–)-1-octen-3-ol (highest sensitivity).

INSECT REPELLENTS AS OLFACTORY RECEPTOR ANTAGONISTS

Pharmacological studies indicate that antagonism of ORs by insect repellents is a phenomenon encountered more often than agonism (Figure 2.2). Deet; SS220; 2U; 3-[*N*-butyl-*N*-acetyl]-aminopriopionic acid ethyl ester (IR3535); 2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester (picaridin); 2,2,8,8-tetramethyl-octahydro-1*H*-2,4*a*-methanonapthalene-10-ol (isolongifolan-8-ol); nepetalactam; nepetalactone; callicarpenal; *para*-menthane-3,8-diol; and a pyrethrin inhibit odorant-activated AaOR2-Orco and AaOR8-Orco.[48,56] Although deet moderately inhibits AaOR2-Orco activation by indole,[48] it has a comparatively strong inhibitory effect on AaOR10-Orco activation by skatole.[49] Deet also inhibits several *Drosophila* ORNs, supporting the theory that insect repellents are broad, and in some cases selective, antagonists of OR activity.[41] IR3535 and picaridin did not exhibit agonist activities for the ORs tested.[56] This observation coupled with the fact that these compounds are structurally different from octenol or indole suggests their interaction with Orco.[48]

A new class of chemical compounds offers promise for the future development of novel repellent formulations. High-throughput screening of human embryonic kidney cells expressing AgORs identified agonist effects of *N*-(4-ethylphenyl)-2-((4-ethyl-5-(3-pyridinyl)-4*H*-1,2,4-triazol-3-yl) thio)acetamide (VUAA1).[15] This complex compound was part of a screening library destined for cancer research. VUAA1 and other analogs[57] exhibited a range of activities on ORs. VUAA1 alone activated Orco as well as ORx-Orco assemblages.[15,58,59] Other VUAA1 analogs antagonized odorant-activated OR complexes.[60] One particular analog displayed a new effect: the *para*-substituted pyridine analog of VUAA1, in addition to activating Orco alone, synergized the activation of OR8 by octenol.[49] Because of their relatively high molecular weights, VUAA1 and its analogs have low volatility and are unlikely to interact with ORs in vivo. Nevertheless, VUAA1 is proof that Orco can be targeted by potentially behavior-modifying drugs.[57]

ACTIVATION OF GUSTATORY RECEPTORS

Although neuromolecular interactions of repellents with ORNs and ORs are well established, neuromolecular effects of repellents on the gustatory system of insects have been documented less often. An early study using a radioactive tracer in feeding tests with *Aedes aegypti* suggested that gustatory sensilla on the proboscis of females were involved in the feeding deterrence observed in the presence of deet.[2] Subsequent behavioral studies further supported the feeding-deterrent effect of deet and other repellents including picaridin, IR3535, SS220, and catnip oil.[61–63] Although these studies showed that the repellents were acting as feeding deterrents and suggested that the gustatory sense was involved at least with deet, evidence was lacking for the nature of neuromolecular effects of these compounds.

The effects of repellents on the GRNs in insects were first shown for deet in *D. melanogaster*.[16] Here, deet activated a GRN sensitive to bitter or aversive compounds in sensilla on the proboscis of adult flies. At least three GRs were involved in the feeding deterrence observed for deet: *Gr33a* (putatively, a required coreceptor[64]), *Gr32a*, and *Gr66a*.

More recently, a GRN housed within sensilla on the labella of *Aedes aegypti* was shown to respond to deet and other repellents including IR3535, picaridin, and citronellal.[3] At least three GRNs (Figure 2.1d) occurred in these sensilla based on the size and shape of the action potentials recorded. A large amplitude action potential was activated by increasing concentrations of salt (NaCl). A somewhat smaller amplitude action potential with a different shape was activated by

sucrose, a feeding stimulant, whereas a small amplitude action potential was activated by quinine, a known feeding deterrent. The GRN activated by quinine was also activated by deet and the other repellents. The potential role of the GRN in feeding deterrence in *Aedes aegypti* correlates well with the earlier behavioral studies.

Although it is convenient to classify gustation and olfaction as separate sensory modalities, GRNs may be activated by high concentrations of volatile chemicals.[65,66] Dethier[65] was the first to show that high concentrations of volatiles activated GRNs in sensilla on the proboscis of the blow fly *Phormia regina*. Later, Städler and Hanson[66] showed responses of GRNs in larvae of the tobacco hornworm, *Manduca sexta*, to food volatiles. Whether or not deet and other repellents are detected in high concentrations by GRNs in *Aedes aegypti* is yet to be determined. However, it is not difficult to imagine that the high concentrations necessary for deet and other repellents to have their effects might be due partly to the involvement of GRNs responsive to them.

CONCLUSION AND PERSPECTIVES

Repellent-treated skin and surfaces do not prevent female mosquitoes from locating their host. It is only at close proximity that airborne deet and other insect repellents disrupt the final behavioral stages leading to landing and subsequent blood feeding (Figure 2.4a). These insect repellents exert their effects by modulating activities of multiple ORs. The observed effects may be the result of interactions between insect repellents and multiple recognition sites on ORs.[67] Both effects occur within a few centimeters of the treated area, which is coated with a high concentration of the insect repellent. These results support the idea that insect repellents confuse mosquitoes rather than elicit evolutionarily selected olfactory pathways for their detection.[68–70] This confusion would result from disrupted brain activities starting within the antennal lobe (Figure 2.4b). The discovery that deet, IR3535, picaridin, and citronellal activate a specific GRN sensitive to bitter or aversive compounds provides a more specific sensory pathway for the feeding deterrence observed for these compounds.

The ORs and GRs are not the only potential transductory sites for repellent action. For example, high concentrations of citronellal, a plant-derived monoterpenoid, activate ORNs in *Drosophila* and elicit avoidance behavior in both flies and mosquitoes.[71] In this case, the mechanism of action of citronellal involves two distinct pathways: transient receptor potential A1 (TRPA1) channels and Orco (Figure 2.2). The multimodal TRPA1 channels[72] exhibit chemosensitive properties similar to the transient receptor potential cation channel subfamily V member 1, which is activated by heat, low pH, exogenous chemicals,[73,74] and neurotransmitters.[75]

Over the last 5 years, several theories on the molecular mode of action of deet and other repellents have been proposed. The discovery that these compounds affect phylogenetically unrelated receptors might explain their broad activities on diverse arthropods. That insects use a different OR gene family than vertebrate G protein–coupled receptors is a strategic advantage for the development of insect-specific repellents. However, the broad activity of chemicals such as quinine as aversive or bitter compounds, and sucrose as a feeding stimulant for both mammals and insects, may present obstacles in the design of selective baits for mosquito management. The goal will be to develop specific insect repellents having a range of activity comparable to kairomones or pheromones as well as possessing their efficacy, that is, affecting insect behavior at low concentrations.

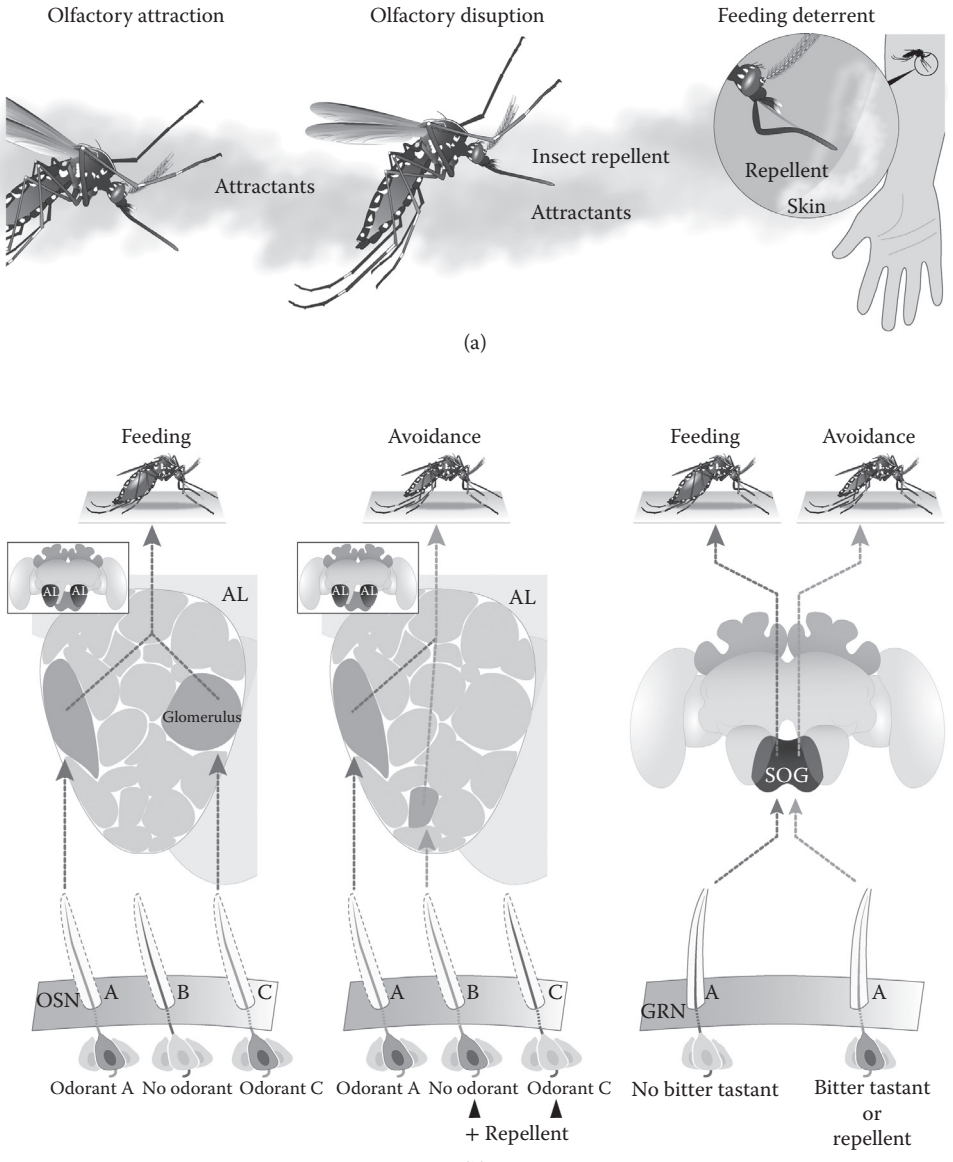


Figure 2.4 (**See color insert.**) Modulation of olfactory and gustatory inputs to the brain leads to behavioral disruption. (a) Kairomones emitted by the host participate along with other sensory cues in attracting mosquitoes. At close range, this attraction will be compromised by high concentrations of insect repellents. The mosquito will go back and forth between these two states until a decision is made to quit engaging the host. On contact with the skin, insect repellents act as feeding deterrents. (b) Attraction is the result of the activity of various brain centers including the antennal lobe (AL). In the presence of attractants, odorants A and C activate olfactory receptor neurons (ORNs) A and C, respectively. The collective activity of ORNs elicits specific activation patterns of glomeruli within the AL. Insect repellents disrupt this pattern by either activation of one or multiple odorant receptors (ORs) or inhibition of other ORs. The resulting disrupted pattern of glomerular activity leads to a confused behavior. Bitter compounds and insect repellents disrupt feeding behavior by exciting gustatory receptor neurons (GRNs) located on the labellum. Sensory output from taste sensilla on the labellum first projects to the subesophageal ganglion (SOG) before reaching higher brain centers. Organotopic and functional organization of taste information in the SOG is not as well understood as in the AL. (From Isono and Morita, *Front. Cell Neurosci*., 4, 20, 2010.)

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CHAPTER 3

How Repellents Work
Neurophysiological and Behavioral Analyses

Zainulabeuddin Syed

CONTENTS

INTRODUCTION

Olfaction in insects is mediated by elaborate olfactory appendages, antennae, and maxillary palps that carry a variety of structures, called sensilla. These sensilla house olfactory receptor neurons (ORNs) in which olfactory receptor (OR) proteins are embedded. A plethora of chemicals originating from skin, breath, plant/nectar, and oviposition sites are detected by these ORNs.[1] Host detection in mosquitoes starts with interactions between odorants and distinct subpopulations of ORs present in the dendritic membrane of ORNs. There are two exciting aspects that make blood-feeding arthropods unique candidates to study olfaction. First, the numerically simple olfactory system (Figure 3.1) at the periphery comprises a handful of ORNs (ca. a hundred or so in bedbugs, ticks, and triatomines, to a few thousand in mosquitoes) housed in simple epicuticular structures termed sensilla. A majority of them are present on antennae and palps, and relatively lower numbers are present on other body parts. Second, distinct and limited range of volatiles that seem to be parsimoniously used in various contexts for releasing distinct behaviors such as attraction and repulsion exists.[6] A recent addition to these advantages includes the availability of genomes of various hematophagous arthropods such as certain species of ticks, triatomines, sand flies, and mosquitoes (www.vectorbase.org). A highly divergent family of seven transmembrane proteins that are functionally and genetically distinct from those discovered in other taxa confers

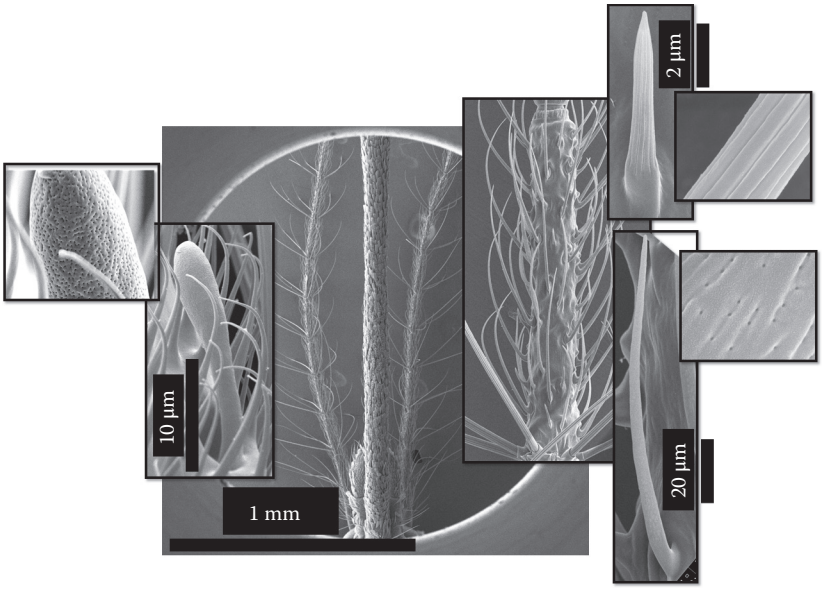

Figure 3.1 Scanning electron micrograph of *Culex* olfactory structures. (Right) Various morphological types of sensilla are seen on an expanded antennal segment. Top right is a grooved peg sensillum (A3 type), and the close-up shows fine groove structures. Bottom right is a blunt tip trichoid sensillum (A2 type) characterized by pores. (Left) Expanded view of the basiconic or peg sensilla and the detailed pore structures. Maxillary palps in mosquitoes are typically adorned with only one morphological type of sensilla that are functionally characterized in *Aedes aegypti*,[1,2] *Anopheles gambiae*,[3]and *Culex quinquefasciatus*.[4]

odorant sensation in the ORNs.[7] Detection of an odorant begins with its binding to an OR that forms a heterodimeric complex with an obligate and highly conserved OR.[8,9] Chapter 2 describes the molecular aspects of olfaction (repulsion) in detail. This chapter essentially reviews seminal discoveries in understanding the neurophysiological and behavioral basis of repulsion in hematophagous arthropods. Such an understanding can be successfully exploited to alleviate the disease burden.

At various points in this book, the term *repellent* will be defined and redefined in different contexts; however, in this chapter it is considered as an airborne chemical that induces electrophysiological response from selected ORNs, resulting in the release of an innate avoidance response.

PERIPHERAL DETECTION OF REPELLENTS

Hematophagous arthropods display robust olfactory behaviors.[10] Exploiting this modality offers exciting opportunities in developing repellents, thereby reducing the frequency of bites and resulting disease burden. Using the olfactory system with its cellular and molecular components as a screening system to isolate and identify biologically active molecules offers a great advantage.[11–13] The olfactory responses from major insect olfactory organs, such as antennae and palps, can be recorded as voltage fluctuations caused by the electrical depolarization of many ORNs[14] on odor stimulation using the techniques of electroantennogram (EAG) or electropalpogram (Figure 3.2). In ticks, where ORNs are localized in the Haller's organs on the first leg pair of tarsi, their "summated" response can also be recorded in a similar manner.[15] Absence of any significant response from these olfactory structures on stimulation with odors will indicate the lack of any biological activity. Early EAG studies suggested compounds eliciting hyperpolarizations (upward voltage deflections) as

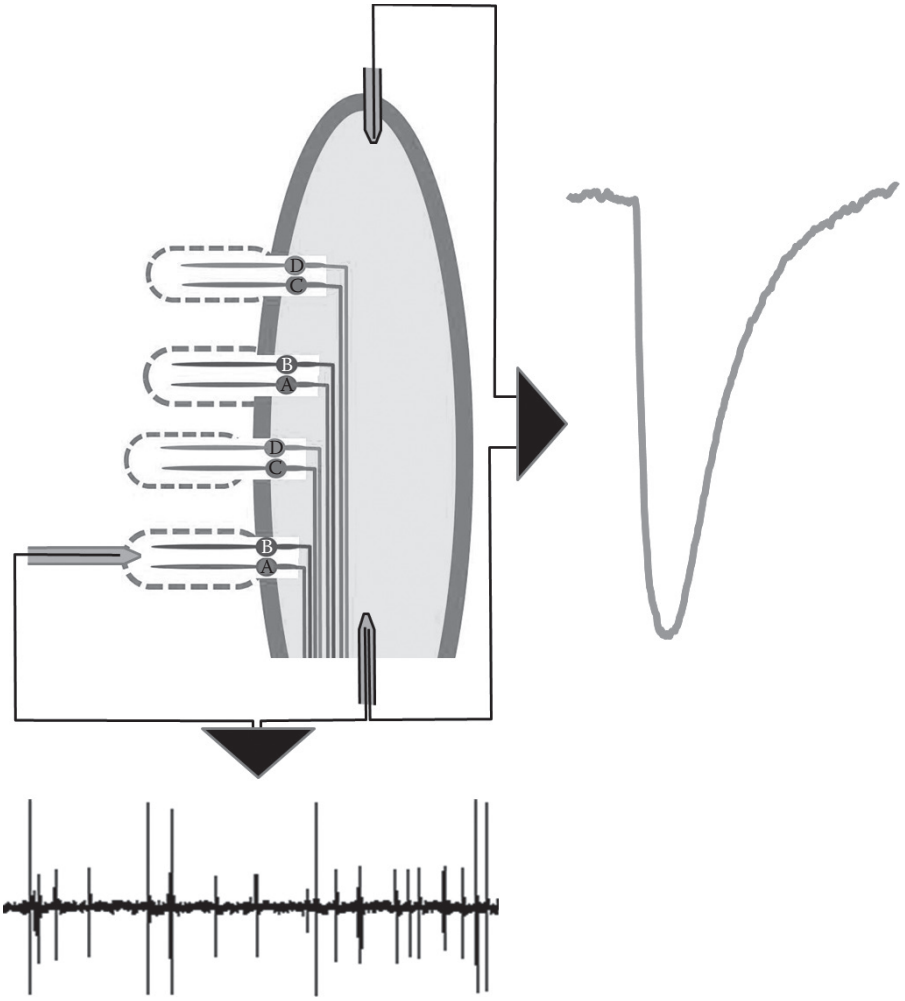

Figure 3.2 **(See color insert.)** Schematic overview of insect antennal structures and possible modes of electrophysiological measurements. Antennae are adorned with many sensillum types, each housing olfactory receptor neuron (ORNs) of various sensitivities as defined by the *olfactory receptors* they express. Approximate summated responses from many/all sensilla can be recorded in the form of voltage deflections, termed electroantennogram (right), or individual ORN responses can be measured by penetrating a single sensillum (bottom).

repellents in subsequent bioassays; however, such suggestions have not been substantiated scientifically. With slight modifications, EAGs have been successfully used to isolate and identify repellents in combination with gas chromatography. This method, described as gas chromatography–linked electroantennographic detection (GC-EAD),[16] has resulted in the isolation and identification of biologically active ligands for a variety of blood-feeding insects. Two recent studies have successfully used the GC-EAD technique in repellent research. A range of organosulfur compounds from garlic essential oil elicited antennal response from *Aedes aegypti*, and a blend of the two most active constituents, namely, diallyl trisulfide and diallyl tetrasulfide, applied to a human forearm provided significant protection from female mosquitoes.[17] In another study, headspace extracts from the widely documented traditional repellent plant *Ocimum forskolei* yielded three behaviorally active constituents, (*R*)-(–)-linalool, methyl cinnamate, and methyl salicylate, that significantly reduced *A. aegypti* attraction.[18]

Despite its potential, the GC-EAD/EAG technique has some limitations. EAG was developed to record responses from male moth antennae that are usually very elaborate and typically dominated by thousands of trichoid sensilla. In addition, these sensilla almost exclusively detect sex pheromones with great sensitivity and selectivity. The sheer abundance and sensitivity of ORNs thus offer unparalleled advantage in moth systems. However, in blood-feeding arthropods there is no such concentration of ORNs or morphological specialization. Overlapping response spectra from various ORNs can easily confound the results. For example, summation of excitatory and inhibitory responses from ORNs of various sensilla[19] and/or the recent evidence of intrasensillar modulation between coinhabiting ORNs can confound the net voltage measurement.[20] This conundrum can be resolved by single-sensillum recordings (SSRs), wherein the action potentials from individual ORNs can be recorded and the specificity in each neuron revealed[21] (Figure 3.2). Olfactory sensilla in blood-feeding arthropods are morphologically diverse but can be broadly classified as trichoid, basiconic, and coeloconic.[22,23] Edward Davis at SRI International performed comprehensive SSRs from a variety of olfactory sensilla in *A. aegypti* antenna, challenging them with at least eight repellents that included *N,N*-diethyl-3-methylbenzamide (deet) and citronellal.[24] The ORNs from A2-II sensillum (short, blunt trichoid) consistently responded to a great majority of these compounds, although deet induced no significant change in the spontaneous activity. Since then, two independent studies have shown A2-II sensilla in *A. aegypti* housing ORNs sensitive to deet.[25,26] A comprehensive SSR analysis performed on all olfactory sensilla types in *Culex quinquefasciatus* identified A1-II (short, sharp trichoid) sensillum that housed two ORNs, one of which responded in a dose-dependent manner to deet.[27] Comparable SSR analysis from *Anopheles gambiae* did not indicate if deet was even tested on ORNs.[4] We found preliminary evidence that deet is detected by an ORN housed in A2-II sensilla in *A. gambiae* (Syed et al., unpublished data).

TECHNIQUES USED FOR IDENTIFYING NOVEL NATURAL LIGANDS

A combination of techniques can be used to isolate and identify novel natural ligands that can potentially replace deet and similar man-made materials. Blood-feeding arthropods live in a rich chemical landscape. Chemicals from and around the host/attractive substrate (headspace odors) can be trapped onto a suitable polymer adsorbent and eluted in a organic solvent; Or, the headspace odors can also be trapped onto Solid Phase MicroExtraction fiber. Extract or the fiber then can be directly injected into the injector of a gas chromatogram and resolved on a high resolution capillary column. Eluted components are split into two after they are resolved at the end of a high-resolution capillary column. A major fraction is diverted onto a clean, humidified airflow bathing a live restrained insect or excised antenna, and the smaller fraction goes to the chemical detector, photoionization detector (PID). Chemical (effluent)-induced physiological (olfactory) responses are measured by the EAG or SSR method, which measures changes in electrical properties. Thus, constituent chemicals of headspace odors are simultaneously monitored via response from a biological detector (insect) and flame ionization detector. Therefore, gas chromatography–electroantennogram and gas chromatography–single-sensillum recording offer a unique platform to use animals as biological sensing elements to identify chemostimuli (Figure 3.3). Compounds that elicit excitatory and/or inhibitory responses can be further screened for their dose–response function to establish their potency. Chemical identity is established by gas chromatography–mass spectrometry and further verified by injecting its synthetic standard and measuring the electrophysiological response. These methods have led to the identification of unique chemostimuli for various hematophagous arthropods: ticks,[28–31] tsetse flies,[32] triatomines,[33] stable flies,[34,35] and mosquitoes.[11,12,36]

Neurons to Neuronal Circuits

Excitation of specific ORNs on the antenna on deet stimulation in *Aedes aegypti*[24–26] and *Cx. quinquefasciatus*[27] has been convincingly demonstrated. How this excitation results in avoidance

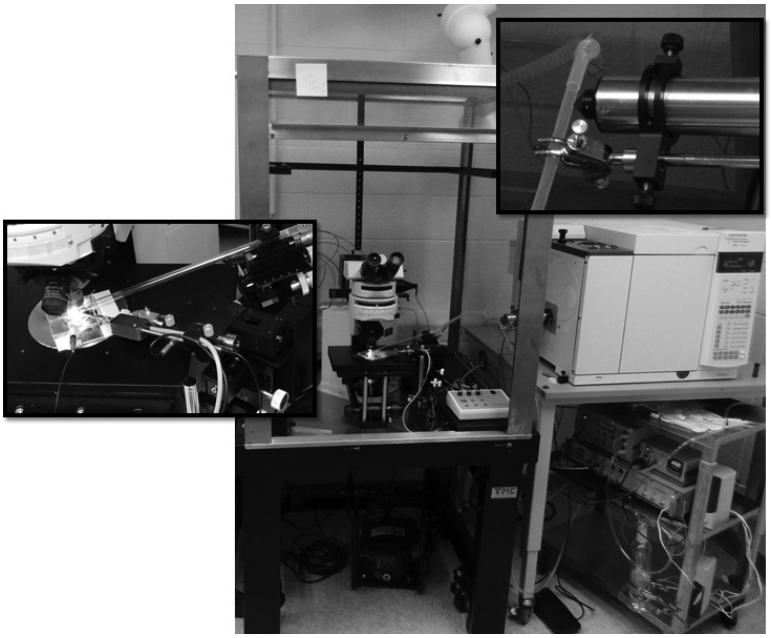

Figure 3.3 A typical electrophysiology rig for recording volatile induced olfactory responses from antenna or a single sensillum in insects, termed as either gas chromatography–linked electroantennographic detection or gas chromatography–single-sensillum recording, respectively. (Right) Close-up view of the assembly that delivers the column effluents as they emerge after the separation on a chromatographic column into the charcoal-filtered humidified airflow bathing antenna. (Left) Close-up view of the restrained insect under high magnification.

behavior still remains a mystery. Few electrophysiological or imaging experiments are done on brain regions such as antennal lobes, projection and interneurons, and mushroom bodies (MBs) or lateral horns (LHs) of hematophagous arthropods. Recent neurophysiological and neuroanatomical studies from *Drosophila* have contributed immensely to understanding how avoidance is manifested at the periphery and higher centers in the brain. In flies, CO_2 elicits strong avoidance.[37] One dedicated ORN in antennal basiconic sensilla responds to CO_2, and the abolition of this ORN through genetic manipulation totally eliminates the avoidance.[38] A further confirmation of the hardwired circuitry was shown by the same group. Artificially activating the CO_2-sensitive ORN with even a nonchemical modality elicited avoidance: flies expressing channelrhodopsin-2 (a blue light–gated ion channel) in CO_2-sensitive ORNs avoided blue light, an otherwise attractive stimulus.[39]

The only other well-studied dedicated olfactory circuitry is involved in the perception of the male-specific pheromone 11-*cis*-vaccenyl acetate (cVA) that elicits excitatory responses from one specific ORN (expressing *Or67d*) in both sexes; but it results in opposite effects in males and females, inhibiting mating behavior in males but promoting it in females. Manipulating the ORN by replacing *Or67d* with different moth pheromone receptors renders these ORNs sensitive to the corresponding moth pheromones and releases complete stereotypic mating responses that mimic the normal response to cVA. Again, activation of a single ORN class was sufficient to mediate behavioral responses.[40]

Stimulus to Behavior

Extensions of the peripheral manifestation resulting in repulsion as dictated by brains are equally exciting. Projection neurons (PNs) sensitive to cVA project to the LH in the brain that receives inputs from olfactory sensory structures responsible for stereotypic behaviors that are innate,[41–43] although

differing opinions exist from moth work.[44] Future anatomical studies in hematophagous insects could provide answers to questions such as the following: Where do the deet-sensitive ORNs converge to form olfactory glomeruli? What interneurons and PNs innervate and exit these glomeruli? Do those PNs project to the MB or LH? Assuming the circuitry is conserved between flies and hematophagous insects, PN projections to the MB or LH will indicate if the deet-induced repulsion is plastic or innate. Electrophysiological single-unit recordings from PNs or high-resolution optophysiological studies in the brain will reveal connectivity. It will be exciting to unravel how the well-studied fly olfactory circuits that underlie repulsion as an innate behavior parallel in blood-feeding arthropods.

Behavioral Output

A variety of bioassays have been developed over recent decades to test candidate chemicals as repellents.[45] Broadly, they can be divided into two categories: in vivo and in vitro. The in vivo assays involve testing a substance that is applied on the skin and compared with the untreated side. Besides posing regulatory constraints, these assays do not evaluate if a biologically active chemical is a repellent by itself. In vitro assays offer the possibility of studying the induced effect of a test repellent on its own and in combination with attractive substrates. Although in vivo assays offer more real-life solutions in providing products that are readily accessible for human use, in vitro assays are critical to extend the neurophysiological analyses of repellent compounds. There are numerous well-studied examples for in vivo evaluations that are reviewed thoroughly in the earlier edition of this book and Chapter 9 of this edition, but recently we designed two novel in vitro assays to test deet-induced behavioral activity in *C. quinquefasciatus*.[27] Both of these assays were designed to test if the mosquito's behavior is directly modulated by the presence of deet in absence of any other attractive chemostimuli. One assay used the propensity of starved mosquitoes (males and females) to seek a sugar meal in the absence of any strong olfactory stimuli. Cotton stubs soaked in sucrose solution were placed inside the cage, and access to these stubs was designed in such a way that adults had to fly through deet-treated filter papers. The second assay was designed to attract host-seeking females with heat at a close range and then exposed to deet in vapor phase as they made a choice to land on the warm black glass tubes. Any possible odor or other sensory stimuli were precluded in both cases. Adults strongly avoided the vicinity of deet. These two assays showed that deet acts as an active repellent. Similar assays exist for ticks, wherein deet was tested in the absence of any olfactory stimuli.[46,47]

Masking and Unmasking of the Masking

Adding deet to an odor cartridge holding the highly excitatory stimulus lactic acid (LA) diminished the net response from LA-sensitive ORN in A3 (coeloconic) sensilla. This led the researchers to infer that "repellents may interact with and inhibit the response to an otherwise strong chemostimuli."[24] This assertion was revived recently by another study wherein delivering deet along with 1-octen-3-ol, an otherwise excitatory stimulus, significantly reduced the response from 1-octen-3-ol-sensitive ORNs in *Aophelos gambiae* maxillary palp sensilla.[48] During our own investigations to study such effects of deet-induced masking of ORN responses, we had an "amazing" set of preliminary data (unpublished) that showed how CO_2 response was modulated in the presence of deet. Proper controls and CO_2 measurements at insect preparation, or the site of electrophysiological recordings, however, revealed that our odor delivery regime was altering the net CO_2 concentration reaching the ORNs. At that point, we decided to quantify the stimulus reaching the physiological preparation. Results were quite unexpected! There was a dramatic reduction in the amount of ligand reaching the preparation if the stimulus cartridge contained deet in addition. Physically separating the two stimuli by placing deet and 1-octen-3-ol in two separate cartridges and connecting their

output with a "Y" to deliver the combined headspace abolished the attenuation. Since then, two independent research groups have confirmed the "masking" as an artifact: John Carlson's group at the Yale University used a PID to show a significant reduction in ethyl butyrate at different doses,[49] and John Pickett's research group at Rothamsted Research, United Kingdom, showed a significant decrease in the *Aedes aegypti* EAG responses to odors released from the stimulus cartridges additionally holding deet.[26] The attenuation was abolished on delivering the stimuli separately. A recent study showed that adding deet to 1-octen-3-ol apparently reduces the amount of free ligand coming out of the stimulus cartridge, although the effects have been termed as nonsignificant.[50] In conclusion, most recent data from the adult mosquito peripheral system confirm[26,5] earlier investigations[24,25] that established that deet acts on specific ORNs and triggers an avoidance response, probably by "labeled line" through a dedicated circuitry.

CONCLUSION AND FUTURE PERSPECTIVES

Detailed neuroethological investigations from the mosquito peripheral olfactory system have provided compelling experimental evidence that deet and related chemicals are detected by dedicated ORNs, which result in releasing innate avoidance behavior.[24–27] Surprisingly, work from the model organism *Drosophila* has produced conflicting interpretations. Studies suggest that deet acts as an "olfactory masking agent"[51]; a gustatory stimulant[52]; an ion channel modulator[53]; and, more recently, a ligand that binds to the specific region of one OR.[50] Availability of transgenic approaches in hematophagous arthropods can allow selective ablation or inducible activation of deet-sensitive ORNs, elucidating if deet-induced avoidance is indeed a manifestation of a dedicated olfactory circuitry. Development of advanced neuroanatomical, electrophysiological, or optophysiological methods that can trace the deet-sensitive ORNs to their glomeruli and higher brain regions will shed light onto circuitry dictating repulsion and related behaviors.

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CHAPTER 4

In Silico Models for Development of Insect Repellents

Kamlesh R. Chauhan and Ulrich R. Bernier

CONTENTS

BACKGROUND

In silico modeling, a common term used to describe computer-assisted molecular modeling, has been used to make remarkable advances in mechanistic drug design and in the discovery of new potential bioactive chemical entities in recent years.[1–3] The goal of this chapter is to focus on new, next-generation computer techniques of molecular modeling to show researchers in the field of arthropod repellents how information on the three-dimensional (3D) structure of small molecules can facilitate the identification, design, and synthesis of repellents through structural activity relationships (SARs) (Figure 4.1). The emphasis is primarily on discussing three recent research approaches of in silico modeling, (1) molecular overlay, (2) artificial neural network (ANN) modeling, and (3) pharmacophore development, focusing on specific sets of arthropod repellents.

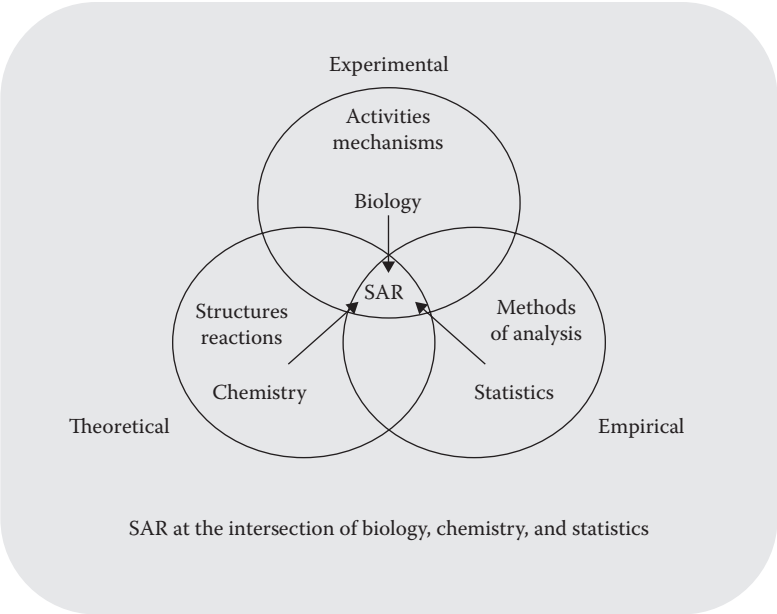


Figure 4.1 Components of structural activity relationships

In silico modeling can provide five major types of information that are crucial for the mechanistic design of drugs and potent new chemical compounds. They are as follows:

1. The 3D structure of a molecule
2. Chemical and physical characteristics of a molecule
3. Comparison of the structure of one molecule with other molecules
4. Graphical visualization of complexes formed between the modeled compound and proteins or other molecules
5. Predictions about how related molecules match the modeled ones, along with an estimate of potency

With the advent of modern computers and graphic techniques, computations and visualization of structures ranging from small to large biomolecules, such as proteins, can be accomplished with greater speed and precision. The graphic tools in modern computers have made it possible not only to visualize the 3D structures of large protein molecules but also to perform interactive, virtual docking experiments between potential drug molecules and the binding sites of proteins. Molecular modeling has now become an inseparable part of research activities that require an understanding of molecular bases of environmental, biochemical, and biological processes. Computational methodologies are routinely being used to make decisions about chemical development and to also perform direct experimental investigations. The current advances in these methodologies allow direct applications ranging from accurate ab initio quantum chemical calculations of stereoelectronic properties, generation of 3D pharmacophores, and performance of database searches to identification of potent bioactive agents.

The discovery of new insect-repellent active ingredients is a complex process with ever-changing new technologies. For example, it still takes about 10 years and, on average, approximately $30 million to bring a new insect repellent to market. Thus, historically, any technology that can improve the efficiency of the process is highly valuable to the commercial industry. In silico technologies are relatively new and have shown remarkable success in recent years, particularly in virtually screening compound databases. These technologies are primarily driven by both cost and time effectiveness of a new active ingredient discovery. Although no model is perfect, regardless of

whatever it represents, the ability to virtually screen hundreds of compounds in a few hours and to construct simulations of 3D protein structures in a computer has pushed these technologies to the cutting edge of discovery of new insect-repellent active ingredients.

The ability of a bioactive molecule to interact with the recognition sites in receptors results from a combination of steric and electronic properties. Therefore, the study of stereoelectronic properties of these molecules can provide valuable information, not only to better understand the mechanism of action but also to develop reliable pharmacophores to aid in the design of more efficient analogs. Quantum chemical computations in modern computers can provide accurate estimates of the stereoelectronic properties of molecules and can thus be used to assess the interaction of potential repellent active ingredients with the receptor.

Developing in silico 3D pharmacophore models and using them selectively as templates for 3D multiconformer database searches to identify new potent compounds are a few of the many other remarkable successes of computational methodologies in recent years.[4] A 3D pharmacophore may be perceived as a geometric distribution of chemical features, such as a hydrogen bond acceptor, a hydrogen bond donor, aliphatic and aromatic hydrophobic moieties, and ring aromatic hydrophobic moieties, in the 3D space that defines the specific biological activity of a molecule. Pharmacophores are generated by multiple conformations from a set of structurally diverse molecules. The generated pharmacophores enable rapid screening of virtual molecules/libraries to identify potent and nonpotent bioactive agents.

ARTHROPOD REPELLENTS

N,N-Diethyl-3-methylbenzamide (deet) has been regarded as the standard mosquito repellent for the past several decades. However, as a repellent for human use deet is not equally effective against all insects and arthropod vectors of diseases.[5–8] In most formulations, it has a short duration of action (not more than several hours) and several disagreeable cosmetic effects, such as an unpleasant odor. Of greater concern is the fact that when it is used in higher concentrations the deeper skin penetration can cause potential toxicity. In addition, deet is a plasticizer that reacts with certain plastics and synthetic rubber.[7]

With increased international travel, illnesses caused by mosquito-borne pathogens, such as malaria, yellow fever, dengue, filariasis, and viral encephalitis, are flaring up all over the globe.[9] One mosquito species that easily adapts to urban conditions, *Culex pipiens*, caused the epidemic of West Nile viral encephalitis in New York City in 1999 that has since spread up and down the eastern seaboard,[10] as well as the rest of North America.

Insect repellents that are completely safe and more effective than current products would be important additions to the armamentarium of tools available to prevent the transmission of arthropod-borne pathogens.

Despite the obvious desirability of finding an effective oral, systemic mosquito repellent, no such agent has been identified.[5,8] Thus, the search for the perfect topical insect repellent continues. This ideal agent would repel multiple species of biting arthropods, remain effective for at least 8 hours, cause no irritation to the skin or mucous membranes, cause no systemic toxicity, resist abrasion and rub-off, and integrate into a greaseless and odorless formulation. Efforts to find such a compound have been hampered by the numerous variables that affect the inherent repellency of any chemical.

All repellents do not share a single mode of action, and surprisingly little is known about how repellents act on their targets.[7] Moreover, different species of mosquitoes may react differently to the same repellent. To be effective, a repellent must show an optimal degree of volatility, making it possible for an effective repellent vapor concentration to be maintained at the skin surface without evaporating quickly so that it loses its effectiveness.

Many factors play a role in how effective a repellent is, including the frequency and uniformity of application, number and species of the organisms attempting to bite, user's inherent attractiveness

to bloodsucking arthropods, and overall activity level of the potential host.[8] Abrasion from clothing, evaporation and absorption from the skin surface, wash-off from sweat or rain, high temperatures, and a windy environment all decrease repellent effectiveness.[8] Each 10°C increase in temperature can lead to as much as a 50% reduction in protection time. The repellents currently available must be applied to all exposed areas of skin because unprotected skin a few centimeters away from a treated area can be attacked by hungry mosquitoes.

APPLICATION OF MODELING METHODS TO REPELLENT DISCOVERY

Structure–activity modeling has also been applied to repellent discovery, with perhaps one of the greater successes being the discovery of 2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester, more commonly known as picaridin, icaridin, KBR 3023, or Bayrepel® (Figure 4.2). This compound was discovered through structure–activity work in the 1980s[10] and marketed as a topical skin repellent in 1998.

Researchers have used 3D quantitative structural activity relationship (QSAR) of deet and related analogs to construct pharmacophores to better understand the structural basis that leads to repellency by these amide compounds.[11–13] Their model was constructed primarily from the protection time data of Suryanarayana and others.[14] Ma and others[11] showed that one could predict repellent duration based on compound structure and specifically that the amide group and attached substituents played a significant role in the experimentally determined repellent efficacy. Using the same data set, Katritzky and others[15] applied Codessa Pro software[16] to develop a QSAR model for the prediction of complete protection time (CPT) from descriptors related to the structural and electronic properties of deet analogs. This work is the foundation for current projects that involve the examination of repellency and toxicity data for subsets of compounds within the U.S. Department of Agriculture (USDA) archive.

In this chapter, rather than discussing the components of in silico modeling we highlight three recent research approaches, (1) molecular overlay, (2) ANN modeling, and (3) pharmacophore development, focusing on specific sets of arthropod repellents.

Molecular Overlay

When QSAR based on numerical descriptors/properties does not work well, comparison of structures instead of calculated descriptors is needed. As repellency of a compound is more of a constitutive property than an additive property, molecular overlay using optimized geometries may be one of the possible ways to resolve this problem. In collaboration with the USDA Agricultural Research

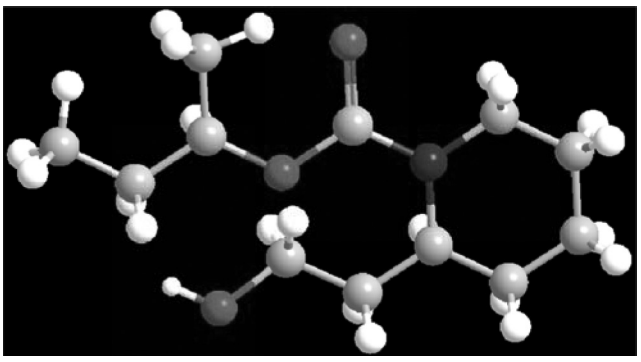


Figure 4.2 (See color insert.) Structure of picaridin (KBR 3023).

Service (through a specific cooperative agreement), Natarajan et al.[17] conducted the molecular overlay approach to evaluate the biological activity of diastereoisomeric mosquito repellents. The molecular overlay in silico modeling was carried out using the biological activity of deet, picaridin diastereomers, and cyclohex-3-enyl 2-methylpiperidin-l-yl ketone diastereomers (Figure 4.3).

Both 1-methylisopropyl 2-(2-hydroxyethyl) piperidine-1-carboxylate [picaridin (R)] and cyclohex-3-enyl 2-methylpiperidin-l-yl ketone (AI3-37220; 220) have two asymmetric centers, and the four diastereoisomers of each compound are known to have differing degrees of mosquito-repellent activity according to quantitative behavioral assays conducted at the USDA. Computational chemistry was used to identify the structural and configurational basis for the repellent activity. Molecular overlay of the optimized geometries of the lowest energy conformers of the diastereoisomers was investigated to elucidate the role of chiral centers in 220 and picaridin. It was found that the presence of a chiral carbon alpha to the nitrogen with the *S* configuration in the piperidine ring is essential to the 3D arrangement of the atoms of the pharmacophore for effective repellent activity.

The comparative mosquito-repellent effectiveness of AI3-37220 and picaridin diastereoisomers against *Aedes aegypti* was determined by applying the compounds to the skin of human volunteers and using the Klun and Debboun (K&D) modules to quantify repellent efficacy.[18] Data presented for the AI3-37220 diastereoisomers are from the study by Klun and others.[19] The combined results of the bioassays of the diastereoisomers of AI3-37220 and picaridin are presented in Table 4.1.

The optimized geometries were superimposed using the overlay command in Chem3D Ultra 8.0 from CambridgeSoft, Cambridge, Massachusetts.[20] Three points (atoms) common to the two structures were considered as the points of superimposition, and the distances between them were set to 0.001 k. Once the two structures were superimposed with respect to the three points, a minimization routine was not applied to avoid the two structures being superimposed at a mean distance with respect to all the atoms in them. If we had used a minimization route, the importance of a substructure or fragment and its effect on the putative pharmacophore could not have been

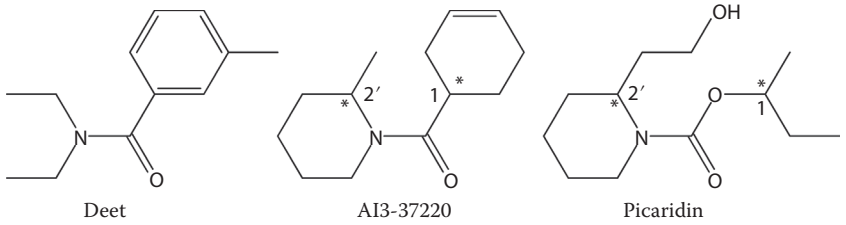


Figure 4.3 Structures of deet, AI3-37220, and picaridin. The diastereoisomeric centers are denoted with asterisks.

Table 4.1 Mosquito Bioassay of Diastereoisomers of AI3-37220 and Picaridin

	Proportion Biting[a]	
Diastereoisomer	**AI3-37220**	**Picaridin**
1*R*, 2′*S*	0.32 b	0.18 a
1*R*, 2′*R*	0.56 c	0.22 a
1*S*, 2′*R*	0.51 c	0.40 b
1*S*, 2′*S*	0.18 a	0.44 b
Racemate	0.45 bc	0.22 a
Control	0.83 d	0.72 c

[a] Proportions followed by different letters are significantly different from one another at $p = .05$.

understood. Hence, after superimposition with respect to the three points the interatomic distances of five atom pairs were obtained and used as a measure of match/mismatch.

The other objective of the study using molecular overlay is to bring out the relative importance of the chiral centers and their positions. The common feature among deet, picaridin, and AI3-37220 is the presence of the amide –N–C (=O)– moiety, and it is interesting that deet is an effective repellent without having any stereo center. This indicates that the amide group may be the putative pharmacophore. These facts raise the following questions:

1. Is one or both of the chiral centers in AI3-37220 and picaridin essential for enhanced repellency?
2. Is the correct absolute configuration at one chiral center more important than the other in determining its efficacy?
3. Which other molecular sites in common for both the achiral deet and the chiral compounds are critical components in the repellency?

In this research,[17] the authors used molecular overlay to investigate the stereochemical structure–activity relationship among picaridin and AI3-37220 diastereoisomers and deet (Figure 4.4). It is clear from the study that most active compounds, picaridin *RS*, AI3-37220 *SS*, and deet, have very similar structural motifs, which leads to a high degree of matching of the relevant parts of a molecule. A sharp contrast to this is the stereochemical similarity/dissimilarity between these three active structures vis-à-vis the less active isomers of AI3-37220 and picaridin.

The most active stereoisomer of a repellent matches better than the least active stereoisomer with deet (standard), which offers explanation why a particular stereoisomer is not an effective repellent. Even diastereomers could be compared. The overlaid structures need not be the conformers of the same molecule. In AI3-37220, the chiral center in the piperidine ring with *S* configuration was identified to be essential for high repellency.

Stereochemical SAR suggests that the critical biomacromolecule responsible for the recognition of the repellent is highly sensitive to the dispositions of the atoms in space. Commonalities in the

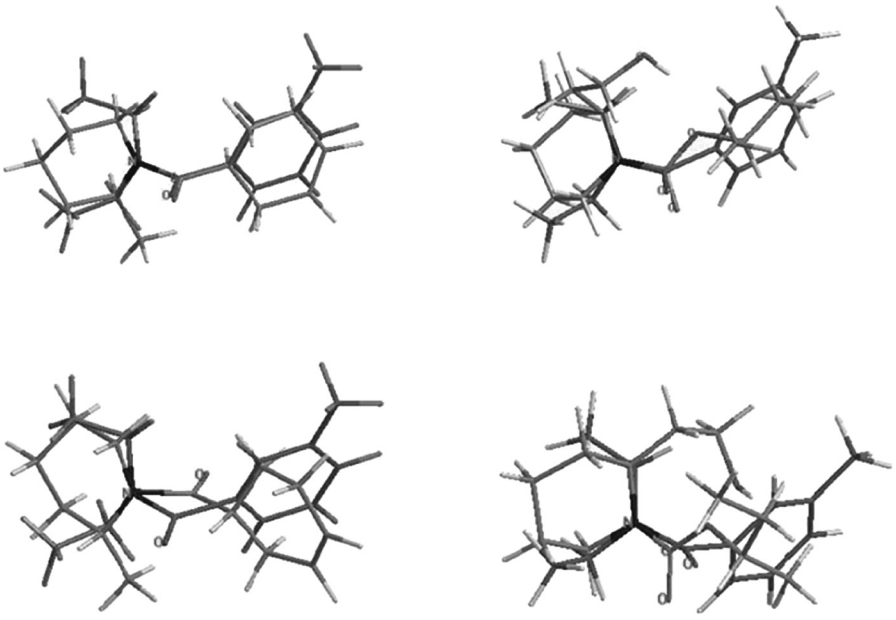


Figure 4.4 **(See color insert.)** Overlay of the most active and the least active diastereomers of AI3-37220 and picaridin over deet.

vicinity of structures within the active space defined by picaridin *RS*, AI3-37220 *SS*, and deet may be useful in the computer-assisted design and synthesis of novel molecules from overlay studies.

Artificial Neural Network Modeling

There is a weakness in the way that repellency data are recorded in the USDA archive, and this impacts the development of structure–activity models. Instead of being reported in days or complete protection time (CPT), the repellent protection times were converted to a five-class system based on CPT, as detailed in Table 4.2. The groupings not only are nonlinear but also tend to equate all superior repellents (class 5) as identical to one another when in fact there can be significant differences in the numbers of days that the compounds are repellent.

Fortunately, ANNs can overcome these limitations and be used to develop models for these types of data. Some of the earliest work with neural networks was done by McCulloch and Pitts in 1943.[21] ANNs can be used for the evaluation of nonlinear data for the development of a predictive model. Thus, a nonlinear data set, such as the class system of CPT data in the USDA archive, can be used to develop a model and predict compound activities based on the compound structures and associated repellent activities that were incorporated into the neural network. Three-layer neural networks with different architectures were applied to the data sets of acylpiperidines in this chapter.

Development of the ANN model was the first step used to predict new repellents. This was accomplished by selecting a set of similarly structured compounds from the USDA archive and then randomly dividing the compounds into training and validation sets. The training set contained approximately 75% of the compounds used to develop the model. The remaining compounds were used as the validation set to verify the accuracy of the model. If there was good correlation between the predicted values (classes in the case of repellents) and the experimentally determined class, then the ANN was used to predict classes for compound structures that were inputted into the model. Some predicted structures were synthesized and evaluated for repellent efficacy by measurement of CPT. Rather than converting these data to classes as was done historically, the actual number of days of protection, or the threshold concentration of protection, was used in the efforts to develop QSAR models.

The initial repellent model for the acylpiperidine data set was developed using 150 out of 200 selected acylpiperidines as the training set for the ANN. A full listing of the compounds (coded by AI3- numbers), structural information, and notation of whether they were in the training or validation subsets can be found in the supporting information for the work by Katritzky and others.[22] This set did not include AI3-35765 or AI3-37220 in the model, but it did contain some compounds similar to those in the structures (Table 4.3, e.g., 4a′-4d′ and others). The archival data used for the initial models in this study were accumulated from compounds submitted as early as 1942 and as late as 1994. The compound structures with AI3- numbers can be found in Table S1 of the supplementary information provided by Katritzky and others.[22] Some of the modeled compounds were from acylpiperidines patented as insect repellents in 1981.[23]

The models for the acylpiperidines were developed with an 8-7-1 architecture, comprising eight initial descriptors as neurons for the input layer, followed by seven neurons in a hidden layer, and

Table 4.2 Five-Class System of Repellents Based on CPT from Treated Cloth and Stockings

Class	Minimum Day	Maximum Day
1	0	1
2	1	5
3	5	10
4	10	21
5	21	—

Table 4.3 Compounds Used for the Acylpiperidine Repellent Study

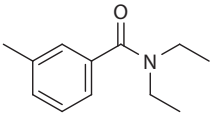
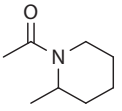
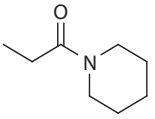
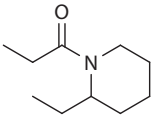
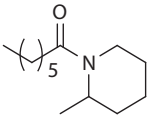
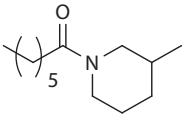
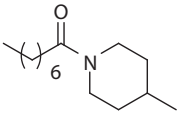
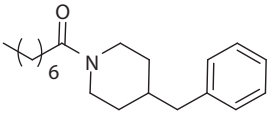
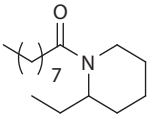
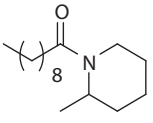
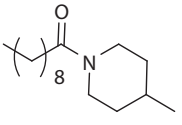
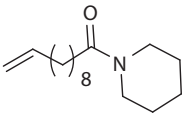
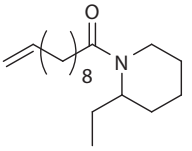
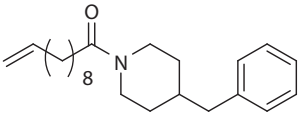
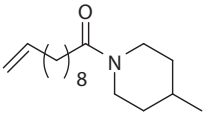
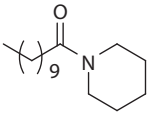
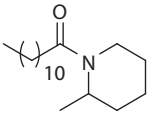
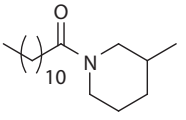
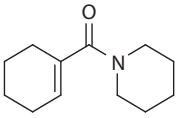
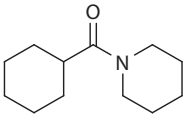
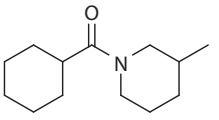
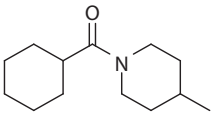
ID	Name	Structure
Deet	*N*,*N*-diethyl-3-methylbenzamide (O-20218)	
4a[a]	1-Acetyl-2-methylpiperidine	
4b[a]	1-(1-oxopropyl)piperidine	
4c[a]	2-Ethyl-1-(1-oxopropyl)piperidine	
4d[a]	2-Methyl-1-(1-oxoheptyl)piperidine	
4e[a]	3-Methyl-1-(1-oxoheptyl)piperidine	
4f[a]	4-Methyl-1-(1-oxooctyl)piperidine	
4g[a]	1-(1-oxooctyl)-4-(phenylmethyl)piperidine	
4h[a]	2-Ethyl-1-(1-oxononyl)piperidine	
4i[a]	2-Methyl-1-(1-oxodecyl)piperidine	
4j[a]	4-Methyl-1-(1-oxodecyl)piperidine	

Table 4.3 Compounds Used for the Acylpiperidine Repellent Study (*Continued*)

ID	Name	Structure
4k	1-(1-oxo-10-undecylenyl)piperidine AI3-39049	
4l[a]	2-ethyl-1-(1-oxo-10-undecylenyl)piperidine	
4m[a]	1-(1-oxo-10-undecylenyl)-4-(phenylmethyl)piperidine	
4n[a]	4-methyl-1-(1-oxo-10-undecylenyl)piperidine	
4o[a]	1-(1-oxoundecyl)piperidine	
4p[a]	2-methyl-1-(1-oxododecyl)piperidine	
4q[a]	3-methyl-1-(1-oxododecanyl)piperidine	
4a′	1-(1-cyclohexen-1-ylcarbonyl)piperidine (AI3-38739)	
4b′	1-(cyclohexylcarbonyl)piperidine (AI3-36324)	
4c′	1-(cyclohexylcarbonyl)-3-methylpiperidine (AI3-36537)	
4d′	1-(cyclohexylcarbonyl)-4-methylpiperidine (AI3-36538)	

(*Continued*)

Table 4.3 Compounds Used for the Acylpiperidine Repellent Study (*Continued*)

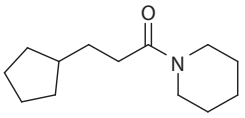
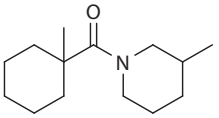
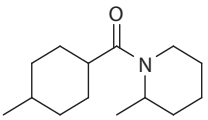
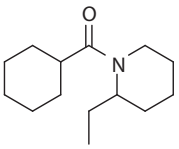
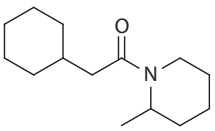
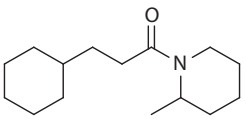
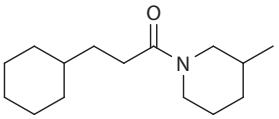
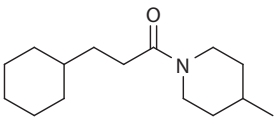
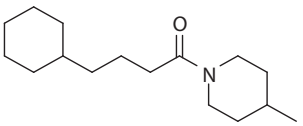
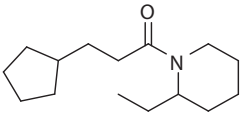
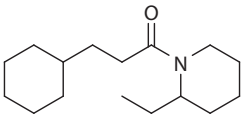
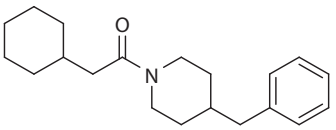
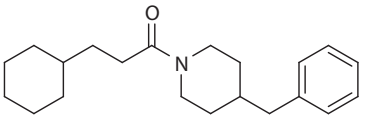
ID	Name	Structure
4e′	1-(3-cyclopentyl-1-oxopropyl)piperidine (AI3-38423)	
4f′[a]	1-(1-methylcyclohexylcarbonyl)-3-methylpiperidine	
4g′	2-methyl-1-[(4-methylcyclohexyl)carbonyl]piperidine (AI3-39012)	
4h′	1-(cyclohexylcarbonyl)-2-ethylpiperididne (AI3-36539)	
4i′	1-(cyclohexylacetyl)-2-methylpiperidine (AI3-37409)	
4j′[a]	1-(3-cyclohexyl-1-oxopropyl)-2-methylpiperidine (AI3-37424)	
4k′	1-(3-cyclohexyl-1-oxopropyl)-3-methylpiperidine (AI3-37425)	
4l′[a]	1-(3-cyclohexyl-1-oxopropyl)-4-methylpiperidine	
4m′[a]	1-(4-cyclohexyl-1-oxobutyl)-4-methylpiperidine	
4n′[a]	1-(3-cyclopentyl-1-oxopropyl)-2-ethylpiperidine	
4o′[a]	1-(3-cyclohexyl-1-oxopropyl)-2-ethylpiperidine	

Table 4.3 Compounds Used for the Acylpiperidine Repellent Study (*Continued*)

ID	Name	Structure
4p′[a]	1-(cyclohexylacetyl)-4-(phenylmethyl)piperidine	
4q′[a]	1-(3-cyclohexyl-1-oxopropyl)-4-(phenylmethyl)piperidine	

[a] Novel compounds.

Table 4.4 Experimental and Predicted Repellency of PMD Analogs

			Number Escaping	
	Experimental	Predicted	Treated	Control
Compound	P^3	P^3	(Mean ± SE)	(Mean ± SE)
1	0.013	0.013	2.5 ± 0.6	0.3 ± 0.2
2	0.2965	0.37	2.2 ± 1.1	0.5 ± 0.2
3	0.0022	0.003	2.7 ± 0.5	0.0 ± 0.0
4	0.4545	0.39	0.0 ± 0.0	0.3 ± 0.2
5	0.0043	0.007	3.5 ± 1.1	0.2 ± 0.2
6	0.0801	0.044	2.2 ± 0.8	0.3 ± 0.2
7	0.4242	0.4	0.8 ± 0.5	0.2 ± 0.2
8	1	0.34	0.0 ± 0.0	0.2 ± 0.2
9	0.0022	0.0037	3.0 ± 0.4	0.3 ± 0.2
10	NA	0.005	NA	NA
11	NA	0.002	NA	NA
12	NA	0.006	NA	NA
13	NA	7.2	NA	NA

Note: NA, not applicable; SE, standard error.

the output of the predicted class as the final neuron (Table 4.4). The input descriptors used to produce the best model were as follows: (1) third-order Kier and Hall index, (2) molecular weight, (3) molecular surface area, (4) total molecular dipole moment, (5) total molecular electrostatic interaction, (6) total number of bonds in the molecule, (7) carbon atom surface area, and (8) nitrogen atom surface area. The resultant ANN model predicted the most efficacious repellents (classes 4 and 5) with 71% accuracy.[22]

With a satisfactory ANN model, structures can be devised and tested in the model to predict their repellent classes. This was performed with just over 2000 acylpiperidine structures. Some of these compounds were tested previously, but many others were novel in that they were not evaluated previously as mosquito repellents. From 2000 predicted compounds, 34 were selected for synthesis: of them, 23 were novel compounds and 11 were chosen from those in the USDA archive. Selection of compounds tested previously allowed for comparison and validation of the current repellent testing methodology with that used decades ago. The repellency data generated for this study were more precise and linear, that is, the repellency was measured in days of protection, rather than put

into classes with nonlinear distributions of protection time. Also, bioassays were conducted with stoichiometrically equivalent amounts of compounds rather than comparison of gravimetrically equivalent amounts, as had been done historically. Generating data based on these changes was necessary for the development of accurate QSAR models.

Summary and Future Work

The repellency class data of a set of acylpiperidines from the USDA archive were used to develop suitable ANN models to predict new repellent structures. Predicted compounds that were not previously examined for repellency along with compounds tested as repellents during the past 70 years were bioassayed for CPT. The results were used to develop a successful QSAR model to predict repellency duration (i.e., CPT), giving excellent correlation with experimental data (Figure 4.5). Compounds such as 4j, 4k, 4o, and 4o′ had durations of repellency three times better than deet.

The approach used to produce successful modeling and prediction of acylpiperidines was also applied to a subset of carboxamides. Because of the greater structural diversity or imprecision in the nonlinear class data, ANN models were not as successful in the prediction of repellents with high efficacy. However, despite the inability of ANN models to produce a QSAR model of carboxamides, about one-third of the carboxamides had a CPT comparable or superior to that of deet and another compound had a minimum effective dose equivalent to that of deet.

Studies are ongoing to evaluate the acylpiperidines and carboxamides against other arthropod species, particularly ticks, and mosquitoes that transmit malaria, such as *Anopheles gambiae* and *Anopheles albimanus*. Traditionally, these mosquito species were more difficult to repel than *Aedes aegypti*. In addition, modeling approaches are being applied to mosquito and housefly adulticide and larvicide data found in the USDA archive.

PHARMACOPHORE MODELING OF *p*-MENTHANE-3,8-DIOL ANALOGS

p-Menthane-3,8-diol (PMD) is an insect repellent that can be either synthesized chemically or derived directly from the steam distillate residue of the leaves of lemon eucalyptus, *Corymbia citriodora citriodora*. It is one of the few natural products endorsed by the Centers for Disease Control and Prevention for topical application to protect against mosquitoes.[24] However, no analytical or quantitative structure–activity studies or toxicological evaluations of PMD have been reported in the literature.

In our ongoing efforts to understand the mode of action of various insect repellents,[25] we have performed a detailed quantum chemical (RHF/6-31G)-based analysis of the stereoelectronic

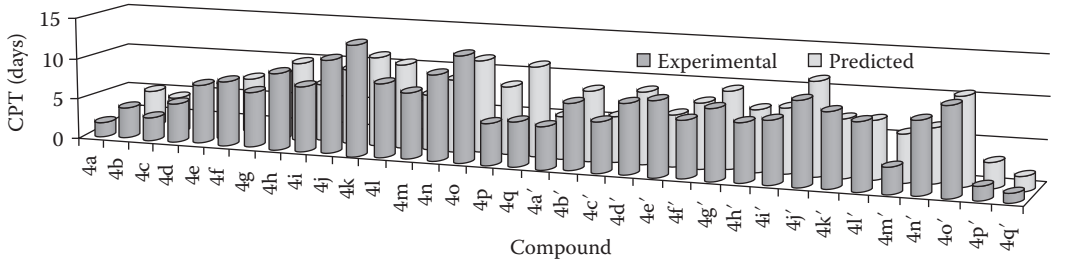
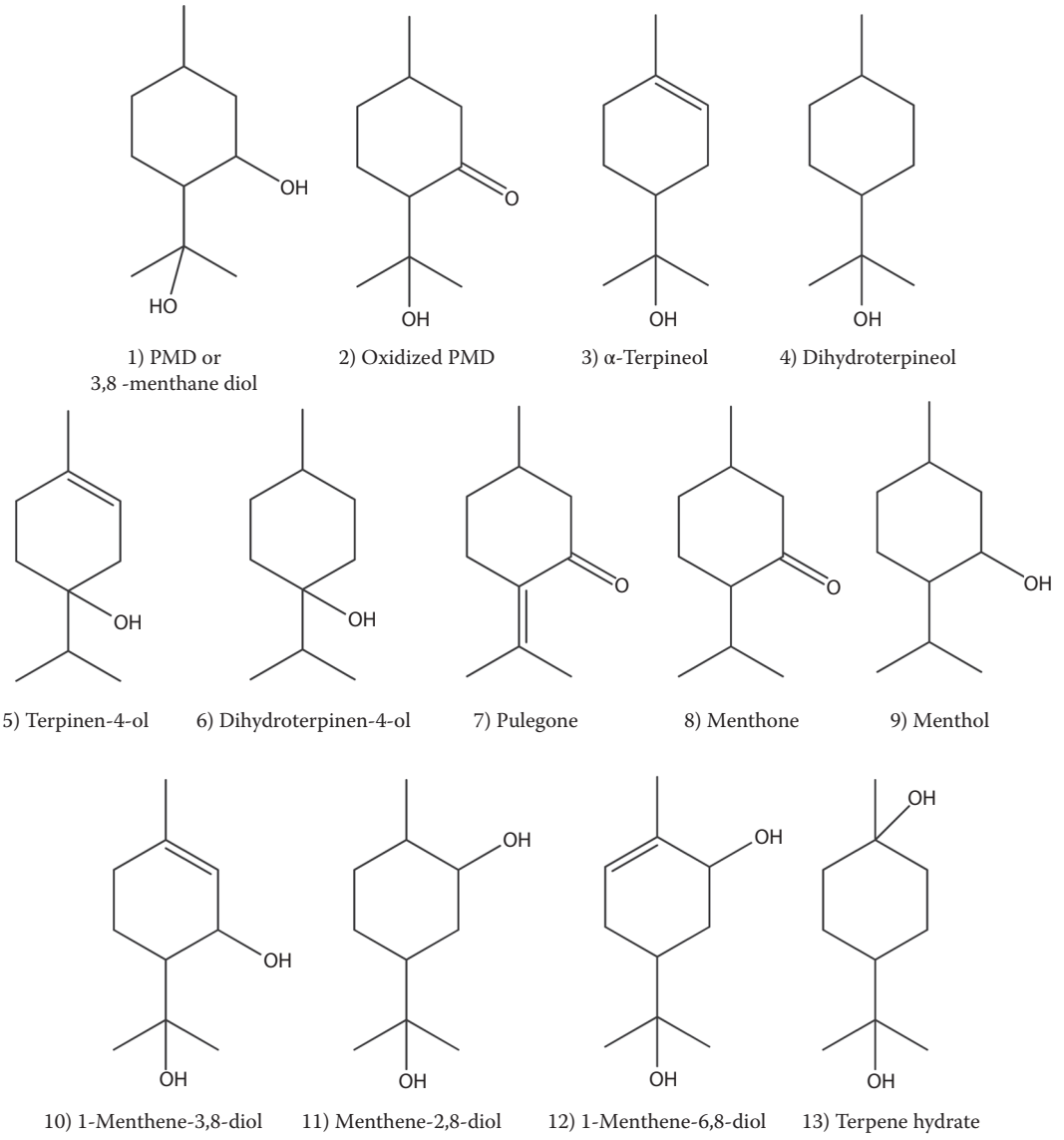


Figure 4.5 Comparison of experimental and predicted complete protection times (CPTs) for the low concentration (2.5 μmol/cm²) of 23 novel and 11 previously tested acylpiperidines (see Table 4.3 for compound structures).

properties of PMD and its 12 synthetic derivatives (Figure 4.6). We have also developed a 3D feature based pharmacophore for repellent activity of the compounds and compared them with other known repellents. Our studies with calculated and experimental observations indicate that a lower aqueous stabilization (favorable lipophilicity) and a larger separation of electrostatic potential energy together with a large localized negative electrostatic potential region by the oxygen atom play a definite role in the repellent activity of these compounds. The generated pharmacophore contained two aliphatic hydrophobic features and a hydrogen bond donor feature that mapped well onto the potent compounds but failed to map onto the less potent analogs. The calculated stereoelectronic profiles and the features of the pharmacophore for the PMD analogs should aid in the design of more effective insect repellents.



1) PMD or 3,8 -menthane diol
2) Oxidized PMD
3) α-Terpineol
4) Dihydroterpineol
5) Terpinen-4-ol
6) Dihydroterpinen-4-ol
7) Pulegone
8) Menthone
9) Menthol
10) 1-Menthene-3,8-diol
11) Menthene-2,8-diol
12) 1-Menthene-6,8-diol
13) Terpene hydrate

Figure 4.6 Structures of *p*-menthane-3,8-diol analogs.

Procedure for the Development of the 3D-QSAR Pharmacophore Model

The 3D-QSAR study was performed using the CATALYST 4.8 software.[26] The algorithm treats molecular structures as templates composed of chemical functions localized in space that will bind effectively with complementary functions on the respective binding proteins. The most relevant biological features are extracted from a small set of compounds that cover a broad range of activity.[27] This process makes it possible to use structure and activity data for a set of lead compounds to generate a pharmacophore representative of the activity of the lead set. The HypoGen algorithm that allows the identification of pharmacophores that are common to the "active" molecules in the training set but absent in the "inactives" are at the heart of the software.[28] Structures of the arthropod-repellent compounds (Figure 4.7) were edited within CATALYST and energy was minimized to the closest local minimum using the generalized CHARMM-like force field, as implemented in the program. Molecular flexibility was taken into account by considering each compound as an ensemble of conformers representing different accessible areas in a 3D space. The "best searching procedure" was applied to select representative conformers within 10 kcal/mol of the global minimum.[29] Conformational models of the training set of 13 repellents were generated, which emphasize representative coverage within a range of permissible Boltzmann population with significant abundance (within 10 kcal/mol) of the calculated global minimum. This conformational model was used for pharmacophore generation within CATALYST, which aims to identify the best 3D arrangement of chemical functions, such as hydrophobic regions, hydrogen bond donors, hydrogen bond acceptors, and positively or negatively ionizable sites distributed over a 3D space explaining the activity variations among the compounds in the training set. The hydrogen bonding features are vector functions, whereas all other functions are points. Pharmacophore generation was carried out by setting the default parameters in the automatic generation procedure in CATALYST (function weight = Z0.302, mapping coefficient = Z0, resolution = Z260 pm, and activity uncertainty = Z3). An uncertainty "D" in the CATALYST paradigm indicates an activity value lying somewhere in the interval from "activity divided by D" to "activity multiplied by D." The statistical relevance of the obtained pharmacophore is assessed on the basis of the cost relative to the null hypothesis and the correlation coefficient.[26,28] The pharmacophores are then used to estimate the activities of the training set. These activities are derived from the best conformation generation model of the conformers displaying the smallest root-mean-square deviations when projected onto the pharmacophore. HypoGen considers a pharmacophore to be one that contains features with equal weights and tolerances. Each feature (hydrogen bond acceptor, hydrogen bond donor, hydrophobic regions, positive ionizable group, etc.) contributes equally to estimate the activity. Similarly, each chemical feature in the HypoGen pharmacophore requires a match to a corresponding ligand atom to be within the same distance of tolerance.[28] The method has been documented to perform better than structure-based pharmacophore generation.[27]

Ab initio quantum mechanical calculations: these were calculated using the RHF/6-31G** basis set of Gaussian 98 package on an SGI Octane workstation:

1. The 3D pharmacophore model for the insect-repellent activity of PMDs showed a good correlation ($R = 0.9$) with experimental data.
2. For repellent activity, the PMDs require a hydrogen bond donor group and two aliphatic hydrophobic features for potent activity. In contrast, the pharmacophoric requirements for deet are a hydrogen bond acceptor group and two hydrophobic groups.[25] Interestingly, the PMD pharmacophore as well as the deet pharmacophore25 map well onto 5-[5 (1-hydroxy-nonyl)-tetrahydro-furan-2-yl]-pentanoic acid a recently reported insect repellent, a novel 18-carbon acid, isolated from samples of greasy gaur hair.[30] Comparison of the PMD pharmacophore with the deet pharmacophore clearly indicates a different kind of mechanism of repellent action of the PMD analogs.

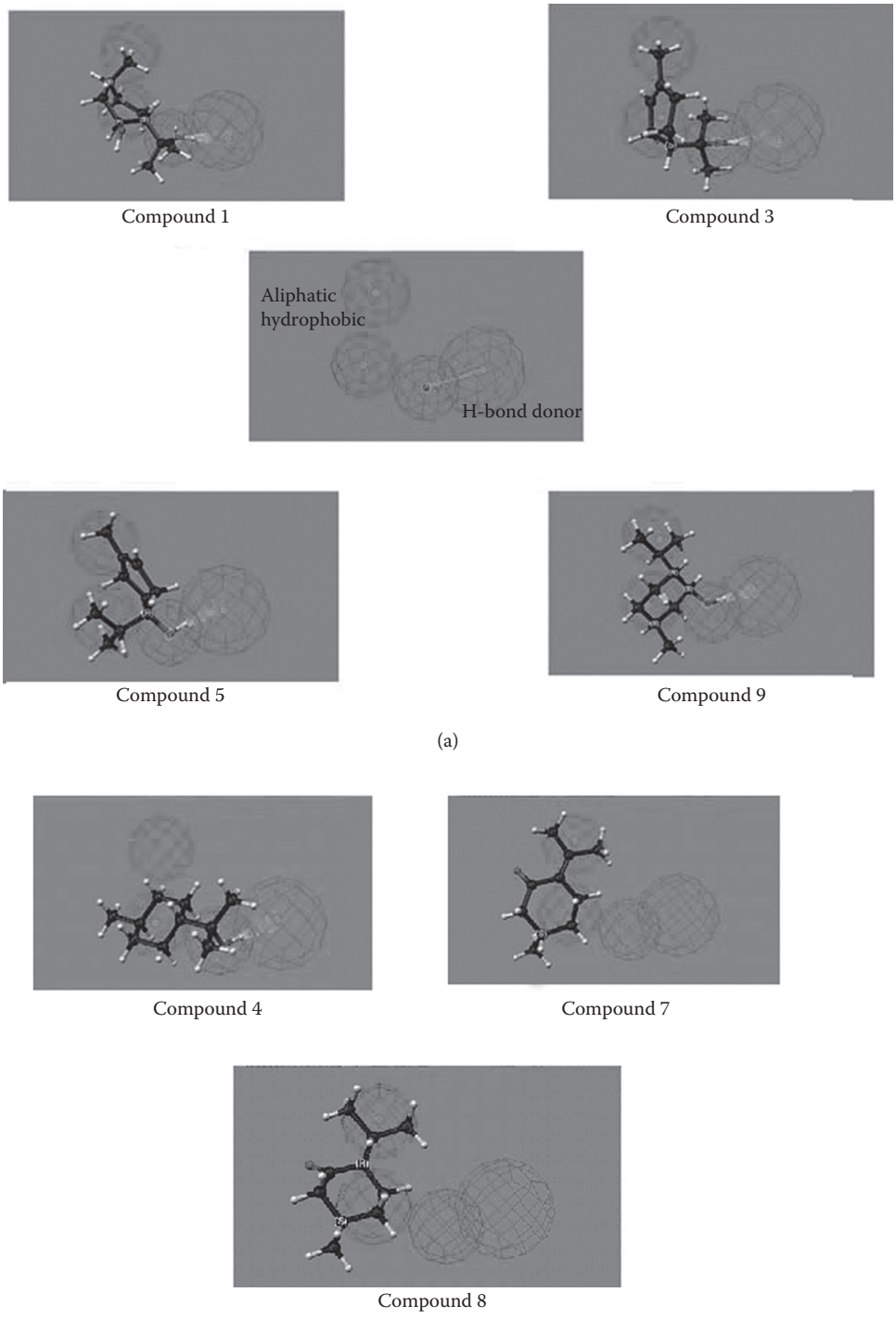


Figure 4.7 **(See color insert.)** Pharmacophore of *p*-menthane-3,8-diol (PMD) analogs. (a) Mapping of the pharmacophore on the PMD analogs with better repellent activity. (b) Mapping of the pharmacophore on the PMD analogs with poor repellent activity.

3. The model has been cross-validated by statistical CatScramble analysis (95% confidence level).
4. Calculated stereoelectronic profiles of the PMDs are consistent with the pharmacophore model. A large, extended electrostatic potential region and weak electrostatic field (hydrophobic) regions appear to favor the potent activity of the compounds.
5. Three of the four new investigational PMDs (10, 11, 12, and 13) map well onto the pharmacophores that are currently being tested.
6. The model is also being used to identify several new potential repellent compounds from virtual screening of the in-house chemical information system database.[31]

It is interesting to note that the localized negative potential region by the amide moiety in the deet compounds is qualitatively linked to their potent repellent activity, with the less potent repellent compounds having a more extended and, therefore, a more diffuse negative potential zone.[32] It appears that a more localized negative potential region in the amide group, as seen with juvenile hormone (JH)-mimic, is consistent with higher protection times. Because the similarity of the negative potential profiles at K10.0 kcal/mol plays a role in the repellent potency of deet analogs, this observation should aid in the design of potent analogs of this class of insect repellents.

Large hydrophobic regions in the molecule appear to be necessary for both recognition and potent repellent activity (Figure 4.8). Hydrophobic effects are the result of the averaged electrostatic interaction of the molecule with its surroundings, solvent, and protein environment. Sites of nonpolar or weakly polar regions in different molecules join together to escape contact with water and minimize the dehydration free energies.[33] Thus, matching the nonpolar regions of ligands with the receptor sites gives a reasonable measure of hydrophobic complementarity and also represents the stabilization of the enzyme–substrate or ligand–receptor complex.

Polarity of a certain region in the molecule can be regarded as being proportional to the electrostatic field. A strong electrostatic field of a molecule attracts molecules having large dipoles, such as water, whereas the weak electrostatic field regions of the molecule do not attract water molecules and are, therefore, hydrophobic.[33,34] Different approaches have recently appeared to theoretically

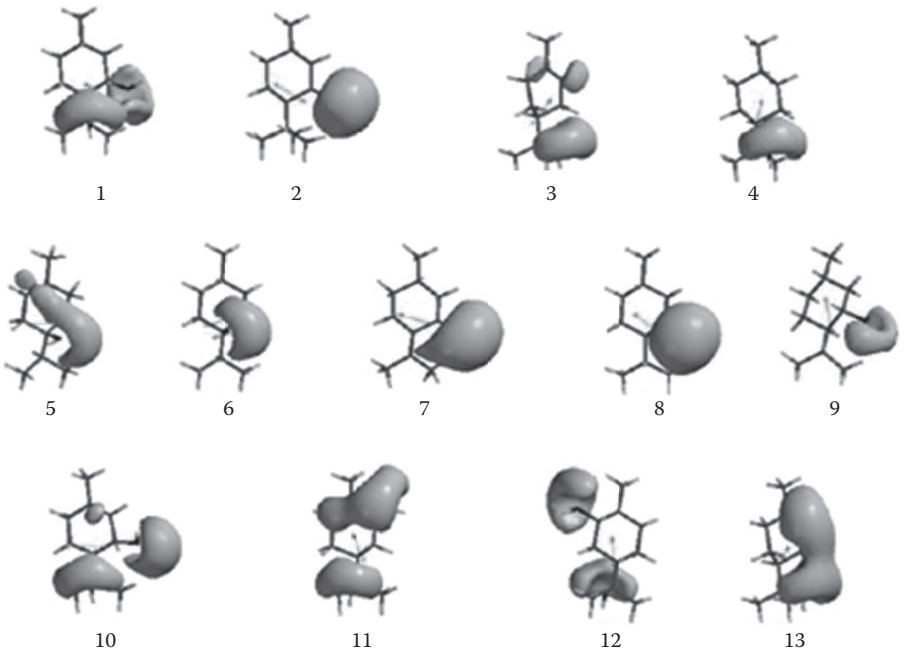


Figure 4.8 **(See color insert.)** Polarity directions and molecular electrostatic potential (MEP) at 20 kcal/mol of *p*-menthane-3,8-diol analogs.

represent hydrophobic interactions in terms of local solute–solvent electrostatics.[32] However, a simple assessment of hydrophobic similarity may be carried out by determining the distribution of charges or electrostatic potentials at different regions on the van der Waals surface of the molecule. The observed low dipole moments of JH-mimic and the deet analogs also correspond to the lipophilic nature of the compounds. Because olfactory sensations of the insects require some degree of lipid solubility,[35] hydrophobicity of the repellents is likely to be an important factor for potent repellent activity.

CHEMICAL FUNCTIONAL REQUIREMENTS FOR ARTHROPOD-REPELLENT COMPOUNDS

Several studies have shown that chemical compounds containing specific functional groups or features are more effective arthropod repellents as measured by the duration of protection.[36,37] Recently, we reported a study[32] of similar analysis of stereoelectronic properties (steric and intrinsic electronic properties) between natural insect JH, a synthetic insecticide (JH-mimic, undecen-2-yl carbamate), and deet and its analogs. Structure–activity studies on JHs have resulted in the discovery of JH-like compounds that mimic the morphogenetic activity of JH with the aim of controlling insect populations.

Understanding the mechanism of arthropod-repellent activity is a major goal of chemists for designing more effective repellents. Because the biochemical steps leading to the desired repellent effect, especially the interaction with the 3D molecular structure of the receptors, are still unknown, various efforts are being made to develop a general structural framework with high probability for repellent activity to guide the synthesis work.[38] The ability of the insect repellents to interact with the recognition sites in receptors results from a combination of steric and electronic properties. Therefore, the study of stereoelectronic properties of insect repellents can provide valuable information, not only to better understand the mechanism of repellent action but also to develop a reliable pharmacophore to aid in the design of more efficient analogs. In addition, a 3D pharmacophore model would be useful to identify the structural requirements for repellent activity that, in turn, could be utilized for 3D database queries to search for proprietary and/or commercially available compounds. Strategies for reducing the abundance and longevity of arthropod vectors of pathogens have been two pronged, centering habitat control (through chemical, physical, engineering, and biological means) and the use of personal protection in the form of insect or arthropod repellents. This chapter also reviews the quantitative structure–activity relationships from currently available scientific data on synthetic and plant-derived insect repellents and how new and effective repellents can be developed using computational methodologies.

Few attempts have previously been made to apply QSAR modeling to repellent activities. This deficiency may be primarily due to the availability of only semiquantitative data on most of the extensive testing that was carried out earlier.[39] One of the first quantitative attempts for measuring molecular properties such as lipophilicity, vapor pressure, and molecular chain lengths was by Suryanarayana and others.[14] Working with 31 insect-repellent compounds, these researchers proposed a QSAR relationship in the form of

$$\mathrm{PT} = a \log P + b \log V_{\mathrm{p}} + c \log \mathrm{ML} + d$$

where PT is the protection time provided by repellent activity; P is lipophilicity; V_{p} is vapor pressure; ML is molecular length; and a, b, c, and d are constants.

Taking into account the paucity of quantitative data on insect repellents and the objectives discussed earlier, repellent structure and electronic properties were initially investigated using quantum chemical methods to determine any functional dependence on protection time as measured

by Suryanarayana and others.[14] The goal of their study was to provide predictive discriminators of insect repellency and a better understanding of the compounds' structure and repellency properties. Although the authors' initial study specifically addresses repellent efficacy, the technique of linking specific molecular electronic properties to biological activity is generally applicable to both efficacy and toxicity studies.

The developmental model of Bhattacharjee and others[32] for structure–activity relationships and generation of pharmacophores was based on the following two approaches:

1. Consideration of electronic and stereoelectronic chemical properties of known arthropod repellents to identify 3D molecular interactions of pharmacophores
2. Consideration of pharmacophores or chemical features of known arthropod repellents to identify 3D pharmacophores with potential repellent activity

DEVELOPMENT OF A NEW MODEL FOR REPELLENT RESEARCH

Chemical Feature–Based Considerations

The factors involved in attracting mosquitoes to a host are complex and not fully understood.[40] Mosquitoes use, at the very least, visual, thermal, and olfactory stimuli to locate a host. Of these, olfactory cues are probably the most important. It has been estimated that 300–400 compounds are released from a human body as by-products of metabolism and that more than 100 volatile compounds can be detected in human breath. Of these odors, only a fraction has been isolated and fully characterized. Carbon dioxide and lactic acid are the two best-studied mosquito attractants. Carbon dioxide released mainly from breath, and also from skin, serves as a long-range airborne attractant and can be detected by mosquitoes at distances of up to 40 m. Lactic acid, in combination with carbon dioxide, and uric acid are also highly attractive.

It is also believed that mosquitoes can sense the host that is the richest source of cholesterol and B vitamins, nutrients that mosquitoes cannot synthesize. Mosquitoes have chemoreceptors on their antennae that are stimulated by lactic acid.[41] It is also speculated that the same receptors may be inhibited by deet-based insect repellents.[42]

As a continuation of the efforts to design and discover new insect repellents from structure–activity relationship studies[32,11] and to better understand the mechanism of insect repellency, a 3D chemical function–based pharmacophore model has been developed.[25] The model connects potent arthropod-repellent activity to compound database searches and aids in the discovery of new repellent candidates. We have used 3D QSAR-CATALYST methodology on a training set of 11 known structurally diverse insect-repellent compounds, including deet, to develop the model whose validity applies to a variety of other arthropod repellents beyond that of the training set.

Electronic and Stereoelectronic Considerations

Because physical–chemical properties of repellents play a significant role in their effectiveness, the role of molecular electronic properties in relation to repellent protection time was also assessed, using a series of deet analogs.[14] Using quantum chemical methods, lowest energy conformations and molecular electronic properties were calculated for 31 amides divided into five different types: (1) *N*,*N*-dimethylamide, (2) *N*,*N*-diethylamide, (3) *N*,*N*-diisopropylamide, (4) *N*-ethyl amides, and (5) piperidineamides. Biological testing of the compounds was performed as reported by Suryanarayana and others.[14] Briefly, a dose of 1 mg/cm^2 was applied to the external surface of a human fist, which was exposed for 5 minutes to 200 *Aedes aegypti* females (aged 5–7 days).

Exposure was repeated every 30 minutes until two consecutive bites were observed, defining protection time as "the time up to the period before the bites."[43]

An examination of the electrostatic potential maps of the repellents at K10 kcal/mol (Figure 4.8), which roughly correspond to the electronic features beyond the van der Waals surface of the molecules, indicated that all repellents have a large, extended negative potential region extending out from the carbonyl group. The electrostatic potential profiles of molecules are considered to be key features through which a molecule fits into a receptor at longer distances and, accordingly, promotes interaction between complementary sites with the receptor.[44] Although this potential characterizes the primary level of interaction with the receptor, there is no apparent relationship with the size or shape of these surfaces to protection time. Regions of positive potentials, the blue-colored regions in Figure 4.7, at the van der Waals surface indicate the electrophilic or acidic sites. Although the location of the most positive potential (deepest blue color) in the repellent molecules is found to be adjacent to different hydrogen atoms on different molecules, the magnitude of the most positive potentials appears to be related to protection time. All compounds that provided protection for at least 2.8 hours had a maximum positive potential in the range of 16.2–21.1 kcal/mol, whereas all compounds with a positive potential higher than 21.1 kcal/mol provided protection for no more than 1 hour.[32] Thus, the intrinsic electrophilicity of the repellent amides appears to play a role in the repellency of a compound. The dipole moment is another interesting electronic property that seems to have a role in repellent activity. This property is the intrinsic polarity of a molecule. Its magnitude is a good indicator of intrinsic lipophilicity or hydrophobicity. In general, the larger the magnitude, the more likely the compound is hydrophilic. In the study conducted by Bhattacharjee and others[11] with 31 repellents, the magnitude of the dipole moment for the most active repellents (protection time observed [PTO] = 3.5 hours) ranged between 3.25 and 3.82 D, an indication that an optimal lipophilicity or hydrophobicity for this class of compounds is necessary for a molecule to be an active repellent. Although the orientation of the dipole moment of the repellents did not seem to have any link to protection time, the negative end of the dipole in these compounds was always observed to be pointing toward the oxygen atom of the carbonyl functional group.

Atomic charges of the compounds seem to have a significant role in repellency. These charges indicate the intrinsic reactive character of the individual atoms constituting the molecules. The magnitude of negative charge on an atom characterizes the nucleophilic nature of the atom, whereas the magnitude of positive charge correspondingly characterizes the electrophilic nature of the atom. In the data set from the aforementioned study of repellents,[11] a low atomic charge on the amide nitrogen atom in compounds having low protection time values was observed. In general, it was observed that the more negative the charge on the amide nitrogen atom, the less the protection time provided by the compound containing the atom.

Significance and Uniqueness of the Methodology

Thus far, no attempt has been made to design insect repellents rationalizing the pharmacophores obtained from similar analyses of studies on stereoelectronic properties. The authors developed a pharmacophore from a training set of deet and its 11 analogs using 3D-QSAR. This was accomplished by utilizing the existing expertise and CATALYST computer software 62 at the Walter Reed Army Institute of Research, Silver Spring, Maryland. The prerequisite for developing a reliable 3D-QSAR model for a novel insect-repellent compound is the correlation of a characteristic and reproducible biological activity to structural information of the respective compound. The conformational model of the compound in the training set has enabled us to use the best 3D arrangement of chemical functions predicting the repellent activity variations among the compounds in the training set. The pharmacophore has also facilitated the search for compound databases to identify new repellent compounds.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

The stereoelectronic properties, similar analysis, and the 3D pharmacophore models in the earlier discussed studies could satisfactorily explain the insect-repellent properties of the evaluated compounds. The pharmacophore model made it possible to search compound databases to identify new repellent candidates. The first investigation on the electronic properties of 31 repellents suggests that the properties of the amide group (N–C=O atoms) in these compounds play a key role in determining the duration of protection against mosquito bites. The substituents attached to the carbon and nitrogen atoms of the amide group together influence the electronic properties of the amide group. Thus, a balance of polarity between the two parts of the molecule seems to be an important contributing factor for potent repellent activity.

The 3D-QSAR pharmacophore study on repellents showed a new computational approach for organizing the molecular characteristics of a set of structurally diverse arthropod repellents to a model that may be both statistically and mechanistically significant for potent repellent activity and may have applicability beyond the bounds of known repellents. The resulting model can also be used to unravel a possible rationale for the target-specific arthropod-repellent activity of these compounds. The chemically significant molecular characteristics distributed on a 3D space generated a pharmacophore that is found to be quite satisfactory in correlating experimental repellent activity with the predicted activity of the compounds (RZ0.9). Potent repellent activity appears to be favored by two aliphatic hydrophobic functions, one aromatic hydrophobic function (aromatic ring), and one hydrogen bond acceptor function in specific geometric locations surrounding the molecular space. The validity of the pharmacophore, which extends to structurally different classes of compounds, allowed us to discover new arthropod-repellent candidates and thereby provided a powerful template for the identification of novel ones. Because the identity of the biological target for arthropod-repellent activity remains unknown, this 3D-QSAR pharmacophore should aid in the design of well-tolerated, target-specific arthropod-repellent active ingredients. The success in discovering new repellent candidates suggests that the 3D-QSAR studies on repellents can not only facilitate the examination of databases to identify new candidates but also be highly beneficial in synthetic efforts to discover better repellents for practical use. Although the process of arthropod-repellent discovery and development is a long and continuous endeavor, in silico technologies can undoubtedly help in reducing the rapidly increasing costs of developing new active ingredients. Molecular modeling techniques using in silico tools are uniquely suitable for integrating new knowledge on molecular structure and repellent activity.

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CHAPTER 5

Can Green Chemistry Provide Effective Repellents?

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CONTENTS

WHY GREEN CHEMISTRY?

Consumers are demanding alternatives to conventional pest management chemicals in every setting, from their houses, gardens, and lawns to the food they buy. Their changing preferences have also been noted in their choice of arthropod repellents that they purchase for use against insects, ticks, and mites. Since 1996, the U.S. Environmental Protection Agency (EPA) has offered an alternative pathway for registration of "biopesticides," which is suitable for many natural or biorational pest control preparations, that is, those related to or based on natural products. The reduced-risk

perspective has resulted in the relatively faster registration of many compounds or extracts that are derived from or designed after natural products. Natural insect repellents have also been registered through the EPA's biopesticides (fast-track) registration process.

What basis do consumers have for their recent interest in more natural product chemistry? The natural products are generally viewed as being safer, specifically to the person using the product and to other nontarget species including pets, livestock, and wildlife. Another aspect of the safety issue is residues that could remain on foods, on clothing, or in the house and lawn, where people of any age could be exposed. The natural products are perceived to have much shorter half-lives in the human environment and on foods, and this perception is largely true.[1] A growing segment of the population is also concerned about effects on nontarget insects, for example, honeybees and butterflies, as well as earthworms and other environmentally important species. Another factor that favors the development of green chemistry for personal protection from insect bites is that product acceptance or compliance in the use of a repellent is lower than optimal in some populations.[2,3] The oily feel or odor can be a negative factor for compliance, as can the ability for *N*,*N*-diethyl-3-methylbenzamide (deet) to dissolve certain synthetic fabrics or fog over plastic watch covers. In addition, the most widely used synthetic repellent, deet, shows up regularly as residues in lakes and streams.[4]

WHAT IS GREEN CHEMISTRY?

The U.S. EPA has provided the following definition for green chemistry: "Green chemistry, also known as sustainable chemistry, is the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances. Green chemistry applies across the life cycle of a chemical product, including its design, manufacture, and use." The EPA, together with the American Chemical Society, has developed a Green Chemistry Initiative, which provides guidance on the processes and products that replace more hazardous processes and products. The term "green" implies that chemicals are of plant origin or that they are broadly more environmentally benign. Many useful natural products for the management of arthropod pests have also been developed from fermentations of bacteria, actinomycetes, or fungi; such products share most of the properties of plant-based natural products and are typically considered to represent green chemistry. Biorational compounds are plant-based chemicals that have been slightly altered or molecules that have been designed after natural products, with resultant properties that are substantially similar to the natural lead compound. Likewise, molecules substantially similar to microbe-based chemicals are also considered to be biorational.

WHAT IS NATURAL?

By the broadest definition, "natural" products can be plant based or microbe based or of animal origin. Even mineral-based materials are naturally occurring, for example, insecticides such as arsenates, arsenites, selenium, thallium, lead, and copper, and fluorides such as cryolite and sodium fluoride. Many of them are not especially safe or biodegradable. Green chemistry indicates that a chemical is natural, but it also implies that it is safe for humans and pets, nontoxic in the environment, and rapidly and fully biodegradable.

How well do the terms natural product and green chemistry align? Does natural mean a material is safe? Many of the most toxic materials known are from natural origins, for example, strychnine and nicotine are plant based; botulinum toxin and ethanol are microbe based; and venoms from spiders, scorpions, snakes, and jellyfish are animal based. These are examples of natural products that are not green chemistry.

Thus, the safety of a natural chemical depends on

1. Chemical structure, not origin
2. Dose or concentration
3. Route of exposure
4. Species that are exposed

WHAT ARE ESSENTIAL OILS?

The oils in some plants provide the "essence" or fragrance of that plant; these oils have therefore been termed "essential oils." They are classified as secondary chemicals by botanists because their functions are often not directly linked to growth, photosynthesis, or reproduction and have been hypothesized to be primarily protective and allow plants to survive and compete better. Essential oils have a rich tradition of use in society over the millennia, not only for their flavor and fragrance but also for protecting against ectoparasites and protecting stored products. Today, they are very widely used in cosmetics and fragrances, for flavoring in foods and beverages, in aroma therapy, and as pharmaceuticals in both traditional and modern medications. Numerous products are currently on the market with registrations for use as insecticides, fungicides, herbicides, antibacterials, and insect repellents.

The chemical constituents of essential oils are primarily terpenes and related compounds (terpenoids) and "green volatiles." The terpenoid fractions, often obtained by steam distillation, are generally more biologically active in protection than the green volatile compounds. The terpenoids will, for the sake of our discussion, pertain to those terpene molecules produced via the isoprene pathway (including hydrocarbon terpenes and various oxygenated forms), as well as those biosynthesized by plants through the phenylpropene/tyrosine pathways. Their structures contain only carbon, hydrogen, and oxygen. Some examples of commonly known essential oils used in flavoring and fragrances are mint, clove, thyme, cedar, cinnamon, rosemary, eucalyptus, and citrus.

HAVE ESSENTIAL OILS BEEN USED AS REPELLENTS?

The essential oil of citronella has been widely used for decades as this natural insect repellent is commercially available. It has been sold for use on humans, as well as for protection of premises, typically by burning yellow citronella candles. Compared to other natural alternatives, citronella oil has been shown to be a relatively weak repellent[5]; but in the United States, it is widely recognized as being safe to use on children and pets. In Canada and the European Union, there are some safety concerns that limit or ban its use. The principal active ingredients in the oil of citronella are monoterpenoids (10-carbon terpenoids), specifically citronellal, geraniol, citronellol, limonene, and methyl isoeugenol (Figure 5.1). In addition to these monoterpenoids, many other plant essential oils or individual monoterpenoids are active ingredients in commercially available natural repellents: rosemary oil, cinnamon oil, mint oils, clove oil, catnip oil, phenylethyl propionate, and lemon eucalyptus oil. Some mammals other than humans have also used defensive chemicals from natural sources (plants and animals) as mosquito repellents.[6]

DOES FOLKLORE HOLD PROMISE FOR GREEN REPELLENTS? IS CATNIP A REPELLENT?

The catnip plant (*Nepeta cataria*) that provides cats with endless intrigue and entertainment has also been reputed to have insect-repelling activity (Table 5.1). In 1964, Thomas Eisner[7] showed that several species of insects, including beetles, hemipterans, a caddisfly, and an ant, moved away from

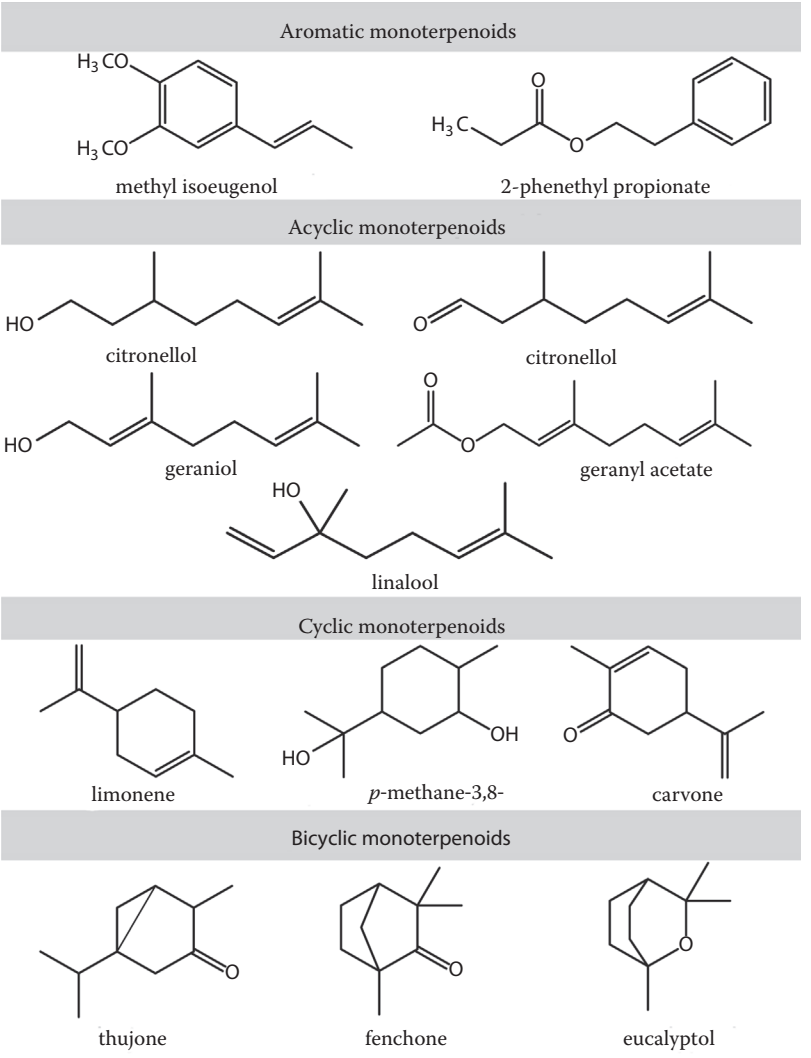

Figure 5.1 Structures of various types of monoterpenoids.

Table 5.1 Summary of Nepetalactone Repellents versus Deet

Nepetalactones	Deet
Broad spectrum of activity	Broad spectrum of activity
Strong spatial repellency	Weak, slow spatial repellency
Strong odor	Light odor
Effective for 1–2 hours	Effective for 6 hours (except for the 7% formulation: 1–2 hours)
Short contact repellency	Long contact repellency

a capillary tube with catnip oil in it; several hemipterans, moths, and a midge did not immediately move away from the capillary of catnip oil.[8] He also showed that the addition of catnip oil to a cockroach cadaver prevented ants from consuming it, and he reported that insect repellency was catnip's reason for existence.[7] In 1999, Peterson et al.[8–10] quantified repellent action for the whole catnip oil, as well as for the two principal isomers *E,Z* and *Z,E* of nepetalactone (Figure 5.2) against German

Figure 5.2 (See color insert.) *Z,E*-nepetalactone (left) and *E,Z*-nepetalactone (right).

cockroaches and compared its repellency with deet, the commercial standard for insect repellency. Subsequent research revealed that several species of mosquitoes were repelled by catnip oil and the individual nepetalactone isomers.[11–15] Their later reports quantified the repellent activity against American and German cockroaches.[16]

The natural repellent citronella has been known for decades to be somewhat effective in repelling insects. When its potency was compared with that of catnip oil and nepetalactones, the oil of citronella showed similar activity. Testing for spatial repellency in a static-air chamber showed that catnip-based oils had much higher potency than citronella, but the two were similar in that the efficacy was strongest initially (15–30 minutes) and then slowly dissipated over 1 hour or 2. In contrast, deet showed minimal spatial repellency, but it increased over a 6-hour period. The difference is explained by the higher volatility of the catnip essential oil, nepetalactones, and citronella oil compared to deet (Table 5.1). The chamber also allowed a measure of contact repellency (by determining the percentage of female mosquitoes that rested on the treated surfaces at the ends of the chamber). Deet showed strong contact repellency throughout the test period, whereas the monoterpene-based oils (nepetalactones and oil of citronella) showed strong contact repellency early, but dissipated over a period of 1–2 hours.

Other investigators have further explored catnip oil or the nepetalactone molecules for their repellent effects. Chauhan et al.[17] compared the two primary isomers of nepetalactone with deet and another synthetic amide. The nepetalactone isomers and the racemic mixture of the isomers exhibited repellency that was comparable to deet in the Klun and Debboun (K&D) module. However, in the testing on human subjects nepetalactones showed 85% biting deterrence, compared to 96% biting deterrence for deet. A study by Bernier et al.[18] showed that catnip oil was a better spatial repellent than deet in a triple-cage olfactometer. However, deet was a better contact repellent against three species of mosquitoes. They also evaluated the repellency of catnip oil and deet in the presence of a human arm or several chemical attractants (lactic acid, CO_2, and acetone).

A group at DuPont, Wilmington, Delaware, hydrogenated nepetalactones to yield two dihydronepetalactones and tested them against mosquitoes, stable flies, and ticks.[19] They also reported that dihydronepetalactone was comparable to deet and *p*-menthane-3,8-diol (Figure 5.1), which is the active ingredient in a commercial botanical repellent. Spero et al.[20] tested liquid and lotion formulations of hydrogenated catnip oil against mosquitoes and blackflies. They found that 15% active ingredient formulations provided 4–8 hours of protection in field tests in Maine and Florida.

Research on the repellency of catnip oil against stable flies was presented by Zhu et al.[21,22] Their use of a wax-based formulation provided for a slow-release longer lasting repellent effect. A subsequent report provided additional results on spatial repellency and the efficacy of catnip oil against stable flies.[23]

Catnip oil is typically composed of 70%–90% nepetalactones (Figure 5.2), which are highly repellent monoterpenoids, and also has caryophyllene (Figure 5.3), which is a sesquiterpene hydrocarbon shown to have little repellency.[24] Two main types of oil of citronella have been reported: the Java type consists mostly of citronellal, with some geraniol, and the Ceylon type is principally

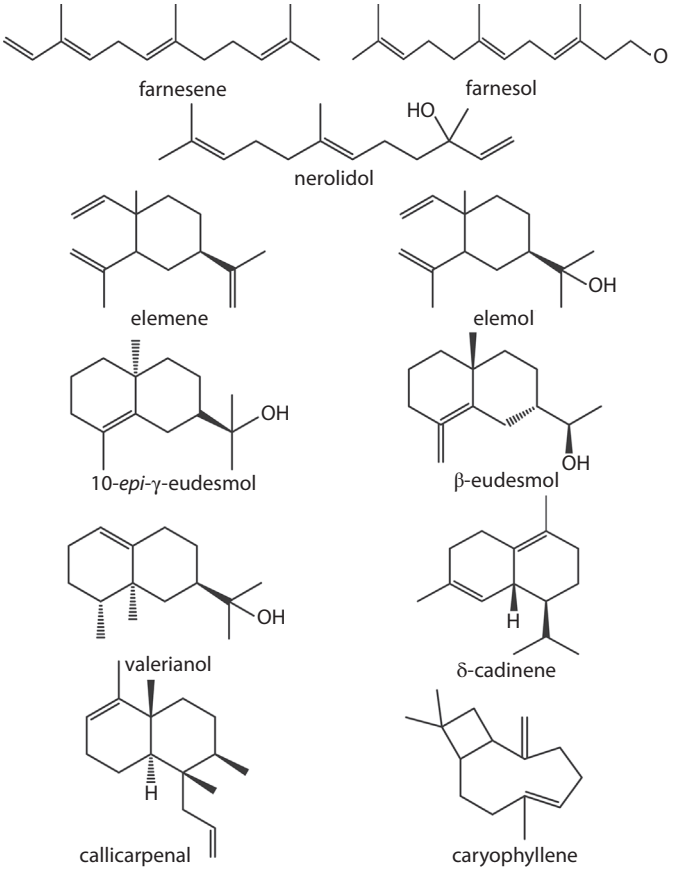

Figure 5.3 Some sesquiterpenoids evaluated for repellency.

composed of geraniol, citronellal, limonene, and methyl eugenol (Figure 5.1). All of these constituents are monoterpenoids that exhibit relatively higher volatility compared to deet, specifically because their molecular weights are significantly lower than deet's and they are not as polar as the amide deet.

ARE OTHER PLANT ESSENTIAL OILS REPELLENT?

Eucalyptus oils have been evaluated for mosquito repellency in Tanzania,[25] and de Boer et al.[26] thoroughly evaluated all of the native plants that are traditionally used in Laos to combat blood-feeding arthropods. Numerous plant essential oils were screened by Barnard[27] on human skin, and the results for efficacy at different concentrations were reported, as well as skin irritation in some cases. Charles Cantrell at the U.S. Department of Agriculture (USDA) Agricultural Research Service Natural Product Utilization Research Laboratory reported on natural repellents from American and Japanese beautyberry leaves,[28] especially the sesquiterpenoids callicarpenal (Figure 5.3) and intermedeol, and the biting deterrence of the oil from the seed of *Jatropha curcas* from India.[29,30] The sesquiterpene isolongifolenone has been isolated from South American *Humiria balsamifera* plants and tested for repellency against ticks and mosquitoes.[31] Considerable research on terpenoid repellents has resulted in the isolation of additional promising individual oils or blends for repelling biting dipterans,[32–34] as well as lice[35] and even structural[36] and agricultural pests.[37,38]

Several other commercially available natural insect repellents also use monoterpenoids from oils of cinnamon, lemongrass, rosemary, and lemon eucalyptus. Other specific monoterpenoids used as active ingredients in repellents are phenylethyl propionate (from peanuts) and *p*-menthane-3,8-diol (from lemon eucalyptus) (Figure 5.1).

ARE OSAGE ORANGES/HEDGE APPLES REPELLENT?

Folklore that catnip repels insects was proven to be accurate; folklore also held that the gnarly green fruit of the Osage orange tree (*Maclura pomifera*) also repels insects and spiders. Its reputed repellency had been reported since the 1800s, but definitive experiments were not conducted until Karr and Coats[39] proved that the repellency of the fruit was significant. Subsequent work showed that the essential oil fraction of the fruit imparted the repellency,[40] although some repellent action against the maize weevil was generated by the two major isoflavones in the fruit osajin, and pomiferin.[41] Analysis of the essential oil of Osage orange revealed over 50 constituents, principally sesquiterpenoids. A total of 14 sesquiterpenoids have been tested for repellency, and a clear indication of one structural requirement was evident: a total of 3 sesquiterpenoids that were hydrocarbons had virtually no repellency, whereas 11 other sesquiterpenoids contained an oxygen atom and were quite active as repellents.[5,15,16,40] Oxygenated sesquiterpenoids included elemol, eudesmol, and farnesol, and three hydrocarbons (elemene, δ-cadinene, and farnesene) were not repellents (Figure 5.3).[42]

ARE REPELLENT SESQUITERPENOIDS FOUND IN OTHER PLANT ESSENTIAL OILS?

The oil of East Indies sandalwood (*Amyris* oil) contains substantial amounts of elemol, β-eudesmol, γ-eudesmol, 10-*epi*-γ-eudesmol, α-eudesmol, 10-*epi*-α-eudesmol, and valerianol (Figure 5.3). Silica gel chromatography, with silver nitrate in some cases, was used to separate preparative amounts of the principal constituents. Five of them were purified and evaluated, and all were found to be strongly repellent.[5] The essential oil of Siamwood, also called Vietnamese pemou wood, was found to be repellent, and two of its major constituent chemicals, nerolidol (Figure 5.3) and fokienol, were also found to be repellent.[4,24,43]

CAN GREEN REPELLENTS ALSO REPEL TICKS?

Sesquiterpenoid repellents have also been evaluated against three species of ticks in laboratory trials.[24,25] Two different laboratories[24,44] studied the repellency of *Amyris* oil and/or elemol against the lone star tick (*Amblyomma americanum*), black-legged tick (deer tick) (*Ixodes scapularis*), or brown dog tick (*Rhipicephalus sanguineus*). Three different climbing bioassays were used to determine the repellent efficacy of the sesquiterpenoids compared to deet. Application rates of 0.2–1.25 mg/cm^2 surface (gauze, cotton, or filter paper) showed that the sesquiterpenoids were nearly equivalent to deet in repellent potency.[44] Witting-Bissinger et al.[45] found that 2-undecanone from trichomes of a wild tomato strain was repellent to ticks. Because it is a nine-carbon molecule, its physical properties more closely align it with monoterpenoids rather than sesquiterpenoids. Recently, Bissinger and Roe[46] have reviewed the topic of tick repellents, including natural ones.

A summary of characteristics of sesquiterpenoid repellents is as follows:

1. Mosquitoes, roaches, flies, and ticks are repelled
2. Weak, slow spatial repellency

3. Light odor
4. Effective for 6 hours
5. Long contact repellency

All of these properties are comparable to those of deet (shown earlier), making the sesquiterpenoids highly promising as natural alternatives to deet. Currently, none are active ingredients in commercially available repellents.

Quantitative structure–activity relationships have been developed for a series of 10 sesquiterpenoids with close structural similarity. Physicochemical parameters were evaluated for their contribution to mosquito-repelling activity. The optimal model that was developed included several electronic properties and vapor pressure as the most important factors in causing repellency, and the most relevant electronic descriptors were polarizability, electrotopological state, and Mulliken populations (of electrons) and the lowest unoccupied molecular orbital.[5,24]

HOW DO REPELLENTS WORK?

Deet is the most widely used mosquito repellent. It was codeveloped by the U.S. military and the USDA as an insect repellent in 1946 and later introduced for public use. Despite several research studies and the wide use of deet for more than five decades, the precise mechanism of repellent action of deet is still being researched, although there are several theories. It seems possible that natural insect repellents act in a similar way, and several hypotheses have recently been reviewed.[47] Curiosity about the mechanism of repellency has resulted in major advances in understanding how insects perceive odors. This topic has recently been reviewed.[48] Several human-specific kairomones have been hypothesized to attract mosquitoes. Potential kairomones include carbon dioxide, lactic acid, and 1-octen-3-ol.[49] The pioneering studies performed by Davis and Sokolove[50] on the mechanism of deet repellency reported that deet blocks the detection of human kairomones. Specifically, Davis and Sokolove showed that carbon dioxide works independently of lactic acid and deet inhibits lactic acid–sensitive neurons.[50] Further studies suggested that there may be several mechanisms of action for deet and other repellents.[51] These studies were supported by behavioral assays that were performed later.

Recent studies on the mechanism of action of deet performed in *Drosophila melanogaster* and *Anopheles gambiae* have shown that *Drosophila* OR47a and OR83b, expressed in antennal basiconic olfactory sensory neurons, were inhibited by deet. Ditzen et al.[52] also showed that two odorant receptors, selective to human body odors in *A. gambiae*, were also affected by deet. In addition, they found that deet inhibited the activity of a 1-octen-3-ol receptor found in the capitates peg on the maxillary palp of *A. gambiae*. Furthermore, deet could affect the movement of *Drosophila* towards food by blocking specific odors related to food, for example, terpenoid-emitting fruits. However, unlike the initial studies by Davis[50] and Ditzen et al.[52] found that deet did not have an effect on carbon dioxide receptors found on the maxillary palp. Therefore, they concluded that deet inhibits odor-evoked currents that are mediated by a select set of odorant receptors and are associated with OR83b, a high-conserved olfactory coreceptor.[51]

Mechanism of action studies were also performed on *Culex quinquefasciatus* using the same experimental setup.[53] *C. quinquefasciatus* showed similar results, whereas deet decreased the neuronal response to 1-octen-3-ol. However, the investigators proposed that these effects were due to experimental error. They suggested that once deet and 1-octen-3-ol were in the same experimental setup, deet would block the effects of 1-octen-3-ol. They suggested that deet would "mask" the effect of human odor and would not directly interfere with the response to a chemical. This masking of 1-octen-3-ol, seen with deet, was also observed with two other common insect repellents (IR3535 and picaridin) in *Aedes aegypti*.[54]

The lack of clarity in understanding how mosquito repellents affect insect activity has contributed to the minimal developmental progress toward new products. Having a precise mechanism of action to study may allow high-throughput methods to be developed to screen large chemical libraries. In addition to potential synthetic compounds, there has been a renewed interest in the ability of green chemicals to be used as mosquito repellents. This is in part because of health concerns or the unpleasant feeling of deet on skin. The use of botanical compounds has previously been reviewed.[24,55] However, the precise mechanisms of action of how these chemicals deliver their repellent effects are not fully understood. Studies have shown that the three monoterpenoids linalool; α,β-thujone; and eucalyptol (Figures 5.1 and 5.3), which were known to be repellent,[53] displayed a dose-dependent stimulation against an odorant neuron in the short trichoid sensillum of *C. quinquefasciatus*. Several odorant receptors from *D. melanogaster* and *Anopheles gambiae* have been characterized using an empty neuron and an endogenous neuron approach.[56,57] Carey et al.[57] found that some of the volatile compounds could be used to attract *A. gambiae* to a host or an oviposition site. The study also evaluated several volatile compounds produced by plants and fruits for their ability to participate in odor reception. In addition, they found that citronellal (Figure 5.1) increased the neuronal firing rate to greater than 200 spikes per second in a particular *A. gambiae* odorant receptor.[57] Interestingly, one *A. gambiae* odorant receptor (AgOr15) inhibited the spontaneous firing rate of six tested terpenoids: (±)-carvone, (±)-fenchone, citronellal, geraniol, linalool oxide, and geranyl acetate (Figure 5.1). Studies also included the investigation of the neuronal location of the odorant receptor, the neuronal processing during odorant receptor activation, odorant combinations, and odorant response based on chemical class and concentration.[56]

HOW ARE REPELLENTS TESTED FOR EFFICACY?

Many apparatuses and experiments have been designed for testing repellents in the laboratory, especially mosquito repellents. They all have advantages and disadvantages, so individual researchers need to select a method that is suitable to best answer the questions they are asking. Testing repellents in the field is more complicated and involves more variables than laboratory trials, but, of course, it has advantages in being similar to "real-world" situations.[58] Maia and Moore[55] have provided several ways to determine mosquito repellency to specifically measure the efficacy of plant-based repellents. In addition, there were special considerations to be determined before testing a mosquito repellent in the laboratory or field.[55] In addition, laboratory testing of human subjects have been designed and are usually termed as arm-in-cage tests. Barnard[27] used this approach to evaluate plant essential oils for their efficacy.

Two distinct types of repellency have emerged important individually or together: spatial repellency and contact repellency. Here, we discuss four methods that have been used in our laboratory for determining the repellency of naturally occurring compounds and essential oils.

Static-Air Repellency Apparatus

Investigations on the efficacy of repellents in a static-air apparatus, which measures 9 × 60 cm (Figure 5.4), allow the measurement of spatial repellency and contact avoidance frequency. A potential repellent is made up in a carrier solvent (acetone or hexane), 1 mL of the solution is applied to a 9 cm filter paper (63.6 cm^2), and the solvent is allowed to evaporate before testing. A treated filter paper (test compound or solvent control) is then secured to each end of the repellency apparatus. Then, 20–25 adult female mosquitoes are added into the middle of the apparatus, and their distribution is recorded at various time points throughout the experiment. Spatial repellency can be calculated as follows: percentage repellency = (number of mosquitoes in the untreated half − number of mosquitoes in the treated half)/(total number of mosquitoes) × 100%.

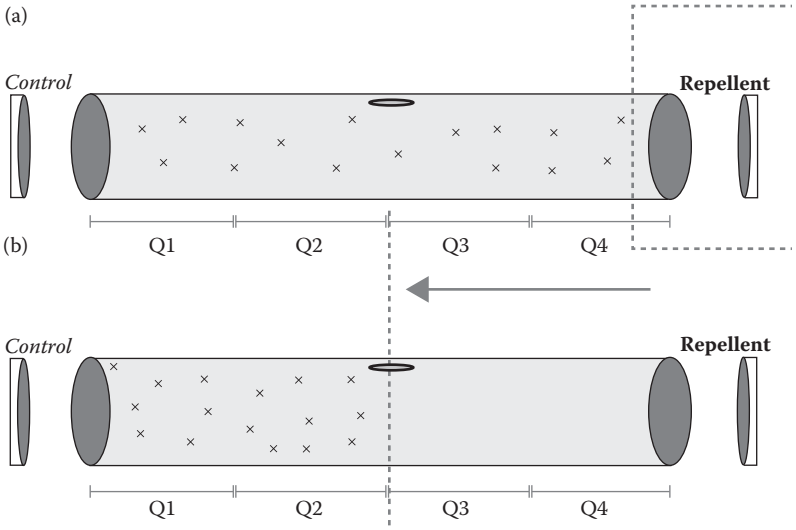

Figure 5.4 Static-air repellency apparatus. This figure shows a control side (9 cm filter paper, no treatment) and a repellent side (9 cm filter paper, with treatment). (a) Normal distribution of mosquitoes (each is displayed as an X) over four quadrants (Q1–Q4) in a "Control" chamber. (b) Spatial repellency causes increased movement of mosquitoes into Q1 and Q2. (Courtesy of G. E. Paluch, Department of Entomology, Iowa State University, Ames, IA.)

In addition to spatial repellency, avoidance frequency can also be measured. At each tested time point, mosquito contact with the treated surface can be monitored. Contact repellency is measured at an instance (recording time point), and if a mosquito (minimum of 1 out of 20–25) is touching or resting on the treated surface it is making contact with the treated surface and therefore not avoiding the treatment. If 100% of the mosquitoes are avoiding the treated surface at this given time point, then the avoidance frequency for this observation time is 1.0. If all are avoiding it at all time periods, then the avoidance frequency for that treatment is 1.0.

Advantages of the static-air repellency chamber are the measurement of mosquito movement in a highly controlled environment, allowance for the quantification of spatial distribution of mosquitoes over time, and determination for residual repellency. Disadvantages of this method include the lack of an attracting source and that some highly volatile and toxic compounds can cause mosquito knockdown or mosquito death.

Klun and Debboun Module

Because adult female mosquitoes seek a host to obtain a blood meal to continue their life cycle, measuring the efficacy of a mosquito repellent in the presence of a host can be beneficial. The American Society for Testing and Materials (ASTM) has developed an attractant: repellency apparatus that was used to design the initial K&D module.[59,60] The K&D module is designed from Plexiglas® and has six adjacent cells (5 cm × 5 cm × 5 cm). Each cell has a 3 cm × 4 cm sliding Plexiglas door that opens toward a human subject. It also has a concave bottom that can easily fit to a subject's thigh.[60] This initial K&D module was the basis for a later in vitro approach, which measured repellency in the absence of a human subject.[61] This updated version includes a Plexiglas blood feeding reservoir on which the K&D apparatus is set. There are six wells in the blood feeding reservoir that would match the six cells in the K&D module. The wells in the blood feeding reservoir are filled with 6 mL of outdated packed human red blood cells supplemented with adenosine triphosphate (ATP). An artificial collagen membrane is used to separate the packed red blood cells from a piece of treated cloth.[61] A disadvantage to the use of packed human red blood cells

is their uneven availability and the protocols needed because of potential blood-borne pathogens. Klun et al.[62] later determined that the use of citrate-phosphate-dextrose-adenine 1, which is used with blood cells as an anticoagulant preservative, then supplemented with ATP, could be an alternative and can stimulate mosquito blood feeding.

Advantages of the K&D module are that it provides a repellency system that allows for the measurement of mosquito contact and repellency as the number of mosquitoes probing and feeding, respectively. Unlike the ASTM model,[59] the K&D module limits this interaction between different chemical treatments; this is achieved by having individual cells separated in the K&D module. The K&D module also increases the number of possible treatments and replicates. Finally, K&D is amendable to multiple sources of attractants (human volunteers, human red blood cells, and artificial blood alternatives). One disadvantage of the K&D module is that it does not allow for the quantification of spatial repellency.

High-Throughput Repellency Apparatus

A novel apparatus was designed and tested by Grieco et al.[63,64] for rapidly screening candidate repellents (Figure 5.5). The specially manufactured assemblies allow for the collection of three

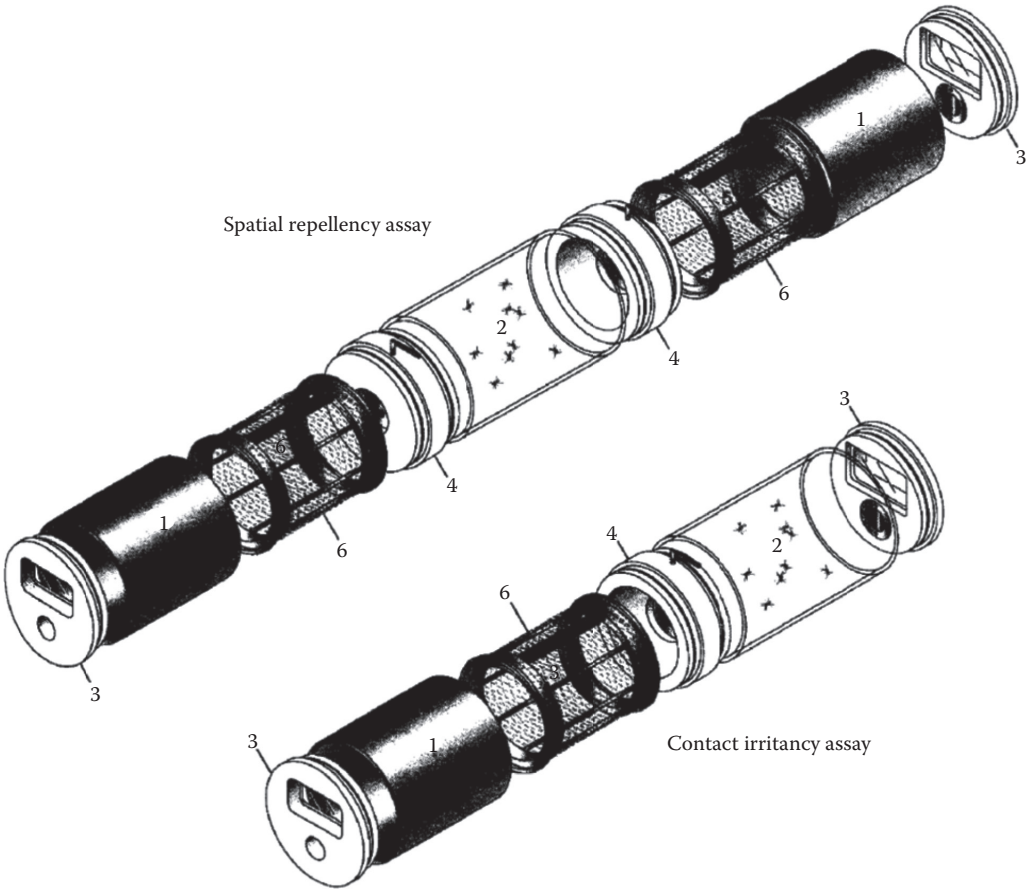

Figure 5.5 Diagram of a novel high-throughput screening apparatus to evaluate the behavior of mosquitoes to tested chemicals. (1) Treatment (metal) cylinder. (2) Clear (Plexiglas) cylinder. (3) End cap. (4) Linking system. (5) Treatment system. (6) Treatment net. (From Thanispong, K. et al, *Journal of Medical Entomology*, 47, 5, 833–841, 2010. With permission.)

types of data, contact repellency (termed contact irritancy assay [CIA]), spatial repellency assay (SRA), and toxicity, simultaneously. It is called a high-throughput screening system because it can provide data on choices that mosquitoes make between (1) a dark chamber treated with a candidate repellent with contact with the treated surface versus a chamber with ambient light (CIA) and (2) a chamber with ambient light versus two dark chambers, one untreated and one with a treated material, but with no contact with the treated material (SRA). Short time periods are adequate to determine choices that the female mosquitoes make. The system has been effectively used for comparing the irritancy and toxicity of synthetic pyrethroids and dichlorodiphenyltrichloroethane for indoor residual spraying (IRS) for malaria control.[65]

Excitorepellency Assay

A larger scale apparatus was designed earlier than the high-throughput screening system,[66] which also addresses the issues that confront IRS programs in the tropics (Figure 5.6). Parallel assays test the preferences of female mosquitoes for dark, treated chambers (with or without contact) versus an untreated chamber (with or without contact) in each case. A more recent version of the testing apparatus has been developed and used.[67] Currently, there are ongoing tests to determine its utility for natural repellents, specifically plant essential oils. The parallel of this system to the situation for IRS for malaria prevention is an advantage of this system.

WHAT ARE THE ADVANTAGES AND DISADVANTAGES OF GREEN REPELLENTS?

For advantages and disadvantages of green repellents, see Table 5.2.

WHAT IS THE FUTURE OF GREEN REPELLENTS?

Many investigators are working on natural repellents, and new ideas and data are being published every year. Several recent reviews are available, including those by Dolan and Panella,[68] Paluch et al.,[69] and Maia and Moore.[55] Spatial repellents are being put forward as the most promising new tactics in the battle against malaria.[70]

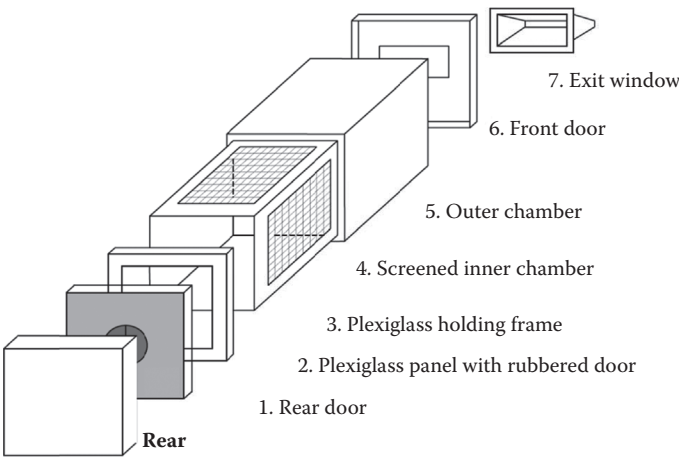

Figure 5.6 Diagram of the excitorepellency apparatus to evaluate the behavior of mosquitoes to tested chemicals. (From Chareonviriyaphap et al., *J. Vector Ecol.,* 27, 250–252, 2002.)

Table 5.2 Advantages and Disadvantages of Green Repellents

Advantages of Green Repellents	Disadvantages of Green Repellents
Generally very safe.	They are "different," so traditional use patterns may need to be updated.
Pleasant odor and feel.	Some have shorter residual action or protection times.
Environmentally friendly and fully biodegradable.	Costs may be higher than synthetic compounds.
New uses are possible (e.g., crop protection).	Supplies of natural products can be subjected to interruption due to crop failures.
Blending of mono- and sesquiterpenoids can provide an optimal blend of spatial and contact repellency.	

Use of natural alternatives to conventional repellents has been slowly increasing. More choices will continue to become available commercially, especially as deeper levels of chemical prospecting progresses. There will be obvious advantages to understanding the mechanisms of action for all repellents, especially the natural ones for which the research has just begun. In addition, quantitative structure–activity relationships need to be further developed for understanding the activity of the molecules and for their predictive value.

CONCLUSION

There are many intriguing opportunities for natural repellents of insects and other arthropods; these repellents include known and novel plant products, individual compounds isolated from the plant products, blends of materials from different plants, and blends of spatial and contact repellents. There are also obvious opportunities for slow-release or delayed-release formulations, as well as for the synthesis of biorational repellent compounds based on variations of effective natural products. As the arthropods continue to be dangerous vectors and determined nuisances, our profession needs to continually provide more effective and affordable repellents.

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CHAPTER 6

Excitorepellency

Ulla Obermayr

CONTENTS

TERMINOLOGY AND CONCEPTS

The use of terminology in the field of repellents aims to create a unique and useful vocabulary to describe mosquito behavior in response to chemicals. As our knowledge of mosquito behavior has increased, the desire to introduce new terms to describe and categorize these behaviors has also increased. Consequently, the field of insect–chemical interactions and insect behavior is rife with terms that attempt to either describe behavioral reactions (effects) or delineate the mediating mechanisms involved (causes). The smaller, more general set of existing terms has been strained

and expanded in an attempt to convey the complex interactions between mosquitoes and chemicals, and terms have sometimes been misused.[1–3]

The term *repellency* is derived from the Latin word *repellere* and has traditionally been used to describe an avoidance reaction, that is, an insect's movement away from a chemical source that is repulsing or deterring.[4,5] "The word repellent has … frequently been incorrectly used."[1] "It is a loose term, looser than we can afford in view of the importance for applied entomology … ."[4] Repellency was suggested to describe effects on the spatial distribution of insects, for example, a surface is considered to be repellent if insects spend less time on it compared to other available surfaces. The term thereby describes an end result, including behavioral reactions, but is not a reaction itself.[4] Dethier et al.[1] refined the definition by distinguishing between two types of repellencies, one that causes an immediate and directed avoidance reaction (*taxis*) and the second one leading to a greater activity (*orthokinesis*) that also reduces the number of mosquitoes on a repelling surface. In 1977, Browne[6] suggested defining a repellent as "a chemical that, acting in the vapor phase, prevents an insect from reaching a target to which it would otherwise be attracted." Such a definition, however, does not include the chemicals that do not act through the vapor phase. Roberts[7] used the term *excitorepellency* to encompass all chemically induced irritant and repellent behaviors. He further distinguished between movements of avoidance resulting from tarsal contact and noncontact actions by classifying chemicals as *irritants* when tarsal contact is required and as repellents when avoidance is elicited through the vapor phase.

The phenomenon of vapor phase–based avoidance is more commonly described as *spatial repellency*. Spatial repellency refers to chemicals that deter mosquitoes at a distance[8] and inhibit their ability to locate a host.[9] Some highly volatile pyrethroid insecticides such as allethrin, transfluthrin, and metofluthrin are also frequently defined as spatial repellents in applications such as mosquito coils, mats, or electric vaporizers that affect mosquitoes by causing knockdown, mortality, repellency, or inhibition of feeding.[10–15]

The variety of terms found in the literature describing mosquito–insecticide interactions is bewildering (Figure 6.1). Muirhead-Thomson[16] regretted that when it came to describing behavioral responses of mosquitoes to residual insecticides "a rather confused terminology has grown up around this basic fact of irritability." If a mosquito settles down on an insecticide-treated surface and manages to take off unharmed before absorbing a lethal dose, it was advised to use the term *protective avoidance*. In case such a behavior was not observed at first exposure but evolved after a certain number of years of being exposed, the term *behavioristic resistance* was suggested. As it is difficult to distinguish natural from developed behavior, Muirhead-Thomson proposed the term *behavioristic avoidance* to cover both.

In 1960, Dethier et al.[1] published their classic paper characterizing chemicals through their modes of action using five basic terms. Chemicals act in different and sometimes multiple ways on an insect. They might cause the insect to stop or rest (arrestant); start or speed up (locomotor stimulant); make an oriented movement toward (attractant) or away (repellent) from the source; or inhibit (deterrent) a certain behavior, for example, feeding, mating, or ovipositioning. It was advised to use the terms attractant and repellent only if an oriented movement to or from the source could clearly be detected. Dethier's definitions provided great progress in the field of terminology and have remained in the entomological literature since then.

The terms repellent, irritant, excitant, and stimulant were commonly used to describe an insect's behavioral response to insecticides, but new terms were frequently introduced while existing definitions were broadened to cover as many aspects as possible. Some of the existing terms, such as repellent and irritant, were considered to be too vague to distinguish between neurotoxic effects and regular sensory inputs,[2] and a new discussion arose regarding the terminology used for insect–insecticide interactions.[3]

Miller et al.[3] updated Dethier's definitions and introduced a new terminology to complement the original terms. Miller used the terms *engagent* and *disengagent* to describe a chemical's effect on

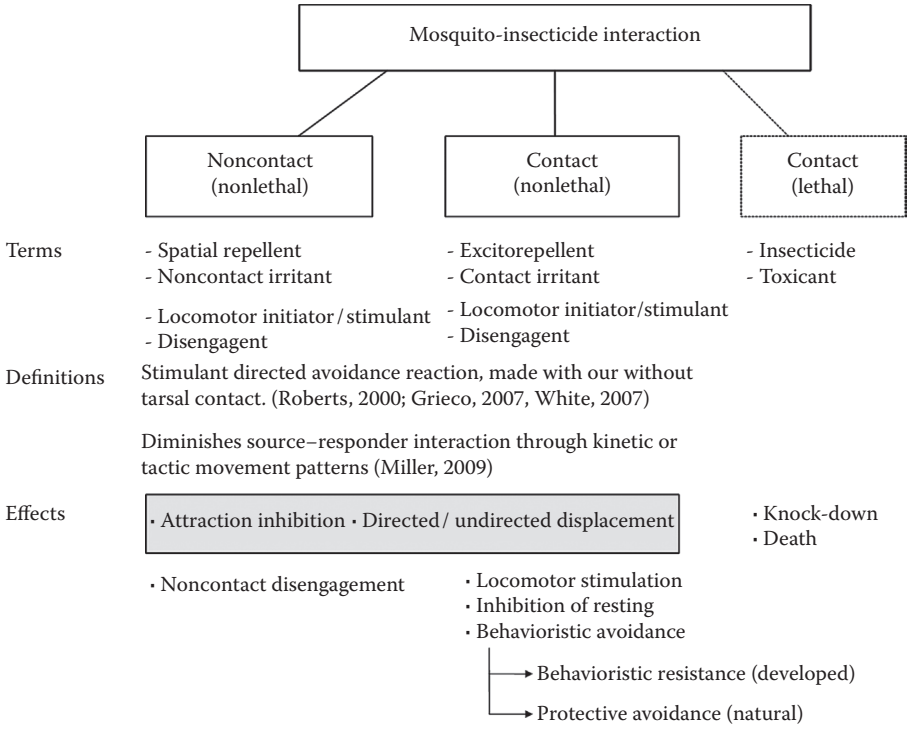

Figure 6.1 Terms and definitions used to describe mosquito–insecticide interactions.

insect locomotion, which can either yield an increase (engagent) or a decrease (disengagent) in the encounters between insect and source. Both effects can be the result of tactic (oriented) or kinetic (nonoriented) movement patterns. Miller disagreed with the definition of contact irritants and spatial repellents, which include an oriented movement away from the source.[7,17,18] Accelerated flight behavior and nonoriented diffusion may also lead to a decrease in encounters, and it was advised to use the terms contact irritancy or spatial repellency only when a steered displacement was clearly detectable.[3] Miller's terminology has not yet gained wide acceptance, and contact irritancy, excitorepellency, and spatial repellency are still the more commonly used terms.[10–14,19,20]

This chapter uses the following definitions:

- Excitorepellency (irritancy): increased locomotor activity after a mosquito has made tarsal contact with an insecticide, resulting in an avoidance reaction
- Spatial repellency: interaction with a chemical in the vapor phase, resulting in an avoidance reaction and reducing an arthropod's ability to locate a host

SHORT HISTORY OF DICHLORODIPHENYLTRICHLOROETHANE

Dichlorodiphenyltrichloroethane (DDT) was first synthesized in 1874, but it was almost 65 years later before its insecticidal properties were discovered by the Swiss chemist Paul Hermann Mueller. Employed by J. R. Geigy, Inc. (Basel, Switzerland), Mueller was searching for new insecticides against clothes moths and carpet beetles when he stumbled across the insecticidal properties of DDT.[21] Samples of the chemical found their way to the United States and the U.S. Department of Agriculture in Orlando, Florida, in 1942. Once its tremendous effectiveness in controlling mosquitoes was demonstrated, DDT was put into service protecting the troops in 1944.

During the latter months of World War II, nearly the total output of United States–produced DDT of 3 million pounds per month was purchased by the military for the control of insect vectors of human diseases.[22] The success of DDT in combating vector-borne diseases is well documented. An epidemic of typhus was controlled and eradicated in Naples, Italy, during the winter of 1943–44, and an epidemic of plague in Dakar, Senegal, was brought under control in 1944.[23] Very favorable and promising results in the control of anopheline vectors of malaria were obtained within the Mediterranean countries and the Far Eastern theater of war.[24,25]

After World War II, DDT became available for general use in 1945. A vast amount of DDT was quickly put to use in agricultural and forest pest control, and initial production grew from 15,000 t in 1945 to more than 35,000 t by 1959.[26]

Indoor residual spraying (IRS) with DDT was the cornerstone of the global malaria eradication effort endorsed by the World Health Organization (WHO) in 1955.[27] Despite tremendous success in eliminating malaria from most of the temperate regions, progress began to slow down in the tropics by the mid-1960s due to several factors: financing became problematic, malaria drug resistance developed, expanding deforestation for agriculture with ill-planned irrigation schemes provided ready habitats for malarial mosquitoes, and DDT resistance became more widespread.[28] The initial euphoria changed to depression, and the public reputation of the magical silver bullet slowly deteriorated.[29] In 1969, the WHO shifted from malaria eradication to longer term disease control strategies relying much less heavily on the use of pesticides.[30] Owing to increased environmental concerns, the United States officially banned DDT in 1972 and its use throughout the world diminished rapidly by the late 1970s.

IRRITANT ACTION OF DICHLORODIPHENYLTRICHLOROETHANE

The emergence and frequent use of the term excitorepellency is closely intermeshed with the history of DDT. During World War II, the main focus of DDT research and development was on finding practical applications. DDT's mode of action and effects on mosquito behavior were not studied until the mid-1940s when its excitant and repellent properties were first observed. Buxton[31] described a certain restless behavior in mosquitoes that had been exposed to DDT, and Gahan et al.[32] and Metcalf et al.[33] reported clear signs of excitation and a subsequent greater attraction to light. However, Buxton considered these observations to be of no practical significance as restlessness or excitation was believed to be an early sign of DDT intoxication that ultimately led to death. Kennedy[4] showed that *Anopheles maculipennis* and *Aedes aegypti* were able to recover from initial DDT poisoning and proposed both lethal and excitant actions of DDT to be considered equally. In contrast to Buxton, Kennedy suggested to use the term repellency to describe DDT's residual effects on mosquitoes after observing that DDT excitation had an impact on the spatial distribution between treated and untreated surfaces, causing a reduction of mosquitoes on DDT-treated sites. In the field, fewer mosquitoes were found in DDT-treated huts[34] and a greater proportion seemed to be able to escape unharmed.[35] The reduced number of mosquitoes inside spray-treated huts was attributed to increased exiting behavior elicited through the contact-irritant properties of DDT. In addition mosquitoes were also found to be deterred from entering treated houses.[36,37] Busvine[38] stated that DDT was able to elicit the following reactions: (1) repellency at a distance, keeping mosquitoes from entering, and (2) contact repellency, which either elicited a greater activity or increased the responsiveness to light, allowing mosquitoes to escape easily. Whether DDT is repellent and the vectors do not enter sprayed houses or whether the vectors enter the houses, become irritated by the insecticide, and quickly exit can often not be determined without lengthy field studies. Despite the fact that avoidance behavior is widespread, it remains poorly understood.[39] It was through these early studies that the importance of DDT's nonlethal excitorepellent effects in contributing to a reduction in host–vector contact and thereby a reduction in disease transmission was first recognized.

RESISTANCE TO DICHLORODIPHENYLTRICHLOROETHANE

Resistance describes the ability of an insect population to tolerate a toxicant to a greater extent than a normal population and to be able to pass this ability on to the next generation.[40] Three mechanisms are responsible for the emergence of insecticide resistance: (1) physiological changes within the metabolism, for example, target-site mutations or accelerated detoxification processes that decrease a toxicant's efficacy or even make it ineffective; (2) morphological changes that reduce the penetration or absorption rate through the cuticle; or (3) behavioral changes that cause a contact avoidance to the chemical.[41] Usually, more than one mechanism is exhibited by a resistant population, for example, mosquitoes' resistance to DDT is based on both physiological and behavioral changes, the latter being the consequence of the irritant and excitorepellent properties of the chemical itself.

The amount of resistance within a certain population depends on the frequency of application, quantity of insecticide, and nature of the target population. Short life cycles and a large number of progeny favor the development of resistance, which explains why resistance emerged rather quickly in mosquitoes when compared to tsetse flies or triatomine bugs. The latter never showed signs of DDT resistance, which can be attributed to a longer life cycle (bugs) and the production of small numbers of progeny (tsetse).[41]

DDT-resistant anophelines were observed as early as 1947 in Italy.[42] In Florida, the use of DDT as a larvicide against the salt marsh mosquitoes *Aedes sollicitans* and *Aedes taeniorhynchus* began on a small scale in 1943 and within a few years DDT was in extensive use. By 1948, only 5 years after its introduction, the target mosquito species had developed high levels of DDT resistance[43] and resistance grew even faster in houseflies.[40]

The situation in Europe and Africa was similar; changes in target vector populations were reported from a growing number of countries. DDT resistance in flies and mosquitoes often emerged within 1–3 years after initial use.[40]

In 1957, the WHO Experts Committee on Insecticides reported evidence-based DDT resistance in 20 insect species of public health importance, with 5 anophelines among them.[44] By 1962, these numbers had dramatically increased to 81 insect species, including 32 anophelines.[45] However, even by 1970 the areas in which DDT resistance necessitated a shift to alternative insecticides represented only 1% of all the areas where DDT could still be successfully used against the malaria vectors.

Despite the development of resistance to DDT in some populations of *Anopheles* mosquitoes, DDT remains generally effective when used for house spraying due to excitorepellency as well as insecticidal effects.[46,47] DDT is still available and used in IRS components of malaria control programs, mainly due to a lack of equally effective and cost-comparable alternatives.[48]

LABORATORY TEST METHODS TO ASSESS EXCITOREPELLENCY

Excitorepellency Test Chamber

In 1957, the WHO Expert Committee on Insecticides[44] stated that "the problem of resistance is growing much more rapidly than measures to deal with it. The stage has now been reached where a greatly accelerated effort … is needed to cope with it." Obviously, there was a strong need for developing (1) new insecticides and (2) standard methods to analyze inherent toxic and behavioral impacts.

Initial bioassays included the use of plastic cones to test the irritability of mosquitoes to DDT-impregnated papers by counting the number of takeoffs within a certain period of time or by determining the mortality rate at 24 hours following the first takeoff.[49] However, counting takeoffs to assess excitant effects produced contradictory results due to a varying degree of mosquito activity in control tests and was therefore considered to be inaccurate.[50] The method was refined and a metal

testing box was introduced, which kept mosquitoes in the dark during testing, thereby reducing overall agitation. Slits in the front side of the box allowed mosquitoes to escape from the treatment area.[50] Using this new assay, the degree of excitorepellency was determined by the number of mosquitoes exiting the test box (lined with insecticide-impregnated paper) and entering a separate (empty) box. In contrast to the cone assay, in which mosquitoes were forced to the insecticide-impregnated surface, the new design was clearly an improvement; but the definition of standardized test doses; the analysis of the outcome; and, most of all, testing the noncontact effects remained challenging aspects.[51]

A new test system was developed to analyze both contact and noncontact-irritant properties. The system included (1) a test chamber that was lined with insecticide-impregnated paper for contact-irritant trials and (2) a slightly smaller screened chamber that could be introduced into the test chamber to hold the mosquitoes during noncontact-irritant trials.[51,52] The front side of each test chamber held an escape slit that ended in an insecticide-free receiving cage (Figure 6.2).

Test chambers were made of metal to keep the mosquitoes in a darkened environment and enable them to orient toward the light that entered through the frontal slit. Before starting, 25 mosquitoes were introduced and given 3 minutes to adapt; then the frontal slit was opened and the number of escaping and remaining mosquitoes was documented every minute for a total of 5 minutes.

This test system offers the opportunity to measure the level of escaping and remaining mosquitoes after both noncontact and contact exposures with the treated surface and thereby provides valuable information on the excitorepellent properties of new chemicals. Data are subjected to a survival analysis (log-rank method) to compare patterns of escaping behavior. The time until 50% and 90% of the test mosquitoes have escaped from the treatment chamber is estimated and compared between different groups of insecticides.[51] Tanasinchayakul et al.[53] devised a smaller, collapsible version of the excitorepellency test chamber, which could be transported to the field for use with wild mosquito populations. The field-compatible version also included an automatic counting system based on a photoelectric sensor that recognized flying objects at a size less than 0.1 mm.

This system has been extensively used to study the behavior of wild-caught mosquitoes in response to standard vector control compounds. Papers treated with 0.04 g/m^2 λ-cyhalothrin caused an escape rate of 95% in *Anopheles minimus* in contact trials. The contact irritancy provided by 2 g/m^2 DDT elicited an escape rate of 67%. Escape responses were weaker in noncontact trials, with 24% avoiding DDT-treated and 19% escaping from λ-cyhalothrin-treated papers.[54] Other trials investigated the avoidance reaction of four malaria vectors from Thailand to deltamethrin. In contact trials, between 70% and 100% of the tested *A. minimus*, *A. dirus*, *A. swadiwongporni*, and *A. maculatus* escaped from papers impregnated with a dose of 0.02 g/m^2. Deltamethrin also showed spatial repellency effects on some *Anopheles* species in noncontact trials. Compared to controls, the proportions of escaping *A. minimus, A. maculatus*, and *A. dirus* were increased between four- and seven-fold.[55] Kongmee et al.[56] investigated behavioral responses of *A. harrisoni* and *A. minimus* to 0.025 g/m^2 bifenthrin and 0.02 g/m^2 deltamethrin in the presence and absence of a host (guinea pig) inside the treatment chamber. Contact irritancy was the main action of both chemicals, and neither elicited a noncontact repellency escape response. Deltamethrin caused a stronger contact-irritant

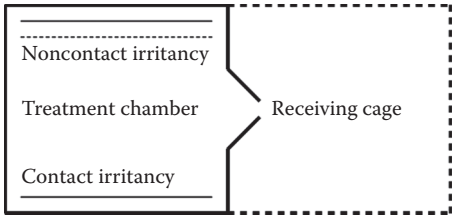

Figure 6.2 Excitorepellency test chamber system.

effect than bifenthrin in *A. minimus*. The presence or absence of a host had no effect on escape responses for *A. harrisoni*, whereas escape responses were delayed in *A. minimus* by both chemicals.

In correlation to real-life conditions, excitorepellency test chambers are a valuable means to predict vector–insecticide interactions inside sprayed houses; however, they are unsuited to gain an understanding of the spatial properties that might impact the level of house-entering mosquitoes.[51] There was doubt if noncontact properties could truly be measured within the small test design as volatiles emitted from the paper might saturate the test space quickly and thereby interfere with the capture of test mosquitoes' responses to an insecticide gradient.[57] This aspect was addressed within the *high-throughput screening system* (HTSS) (see the section "High-Throughput Screening System") and in field trials with experimental huts (see the section "Field Tests Using Experimental Huts").

High-Throughput Screening System

The HTSS is used to test the effects of new chemicals on the behavior of adult mosquitoes, including contact irritancy, spatial repellency, and toxic actions. The modular device (Figure 6.3) uses different arrays of aluminum (test) and Plexiglas (control) cylinders, depending on the objective of the assay.[57]

In contact-irritant assays, a test cylinder lined with a treated net is fixed to a darkened control cylinder. A valve between test and control unit is closed and 10 test mosquitoes are released into the treated cylinder. After an adaptation period of 30 seconds, the valve is opened and the distribution

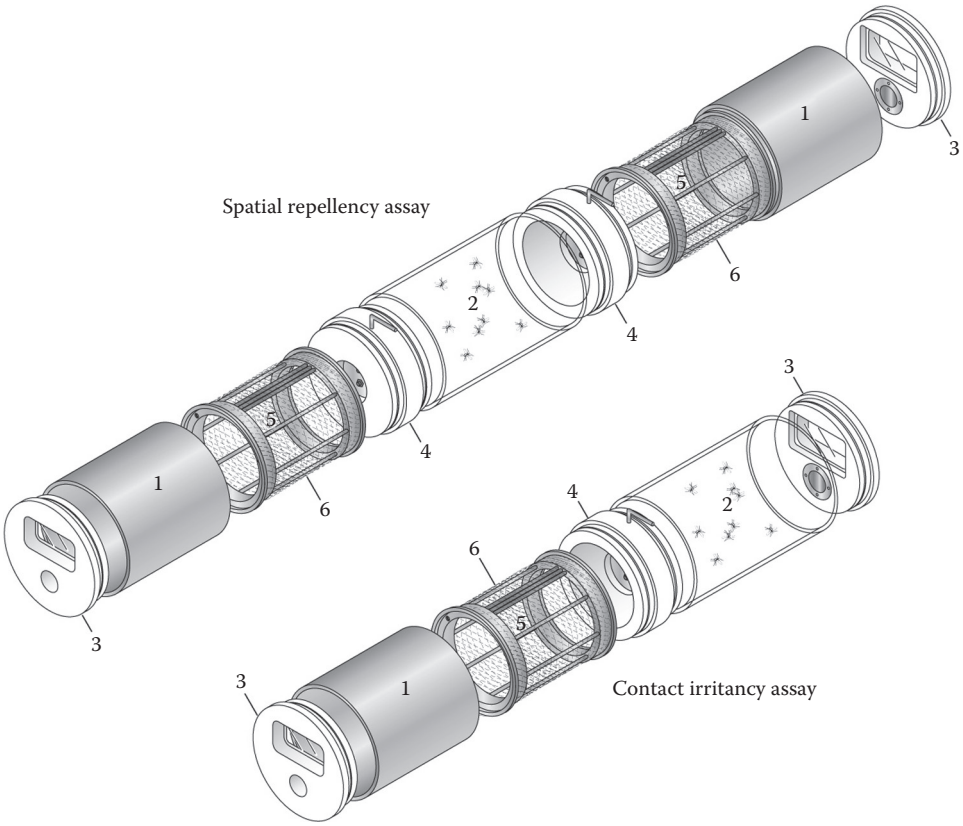

Figure 6.3 The high-throughput screening system according to Grieco et al.. (From Grieco et al., *J. Am. Mosquito Contr.*, 21 (4), 404, 2005.)

of the mosquitoes between the two compartments is recorded after 10 minutes. Individuals found in the control cylinder at the end of the test represent the proportion of escaping mosquitoes. Their numbers are compared to control trials (with ethanol-treated nets) by using the Mann–Whitney U test to examine the level of contact irritancy provided by a test chemical.

In spatial repellency assays, a metal test cylinder containing a treated net and another containing a solvent-treated net are connected by a clear cylinder that is placed in the middle. The valves of the intersections are closed and 20 mosquitoes are introduced into the clear central cylinder, which is darkened by opaque felt. The end caps of the test cylinders are not covered to allow light to enter the system and help mosquitoes to orient. After an adaptation period of 30 seconds, valves are opened and the distribution of test mosquitoes among the test chamber (with treated net), central chamber, and control chamber (with solvent-treated net) is recorded after 10 minutes. With these numbers, a spatial activity index (SAI) can be calculated[58] as follows:

$$\mathrm{SAI} = \left[\frac{(N_C - N_T)}{(N_C + N_T)}\right] \times \left(\frac{N_m}{N}\right)$$

where N_C is the number of mosquitoes inside the control chamber, N_T the number inside the test chamber, N_m the number of mosquitoes in both metal chambers, and N the total number of mosquitoes inside the system. The SAI varies between −1 and 1, with 1 indicating a high level of spatial repellency and −1 indicating no spatial repellency.

Toxicity is evaluated using a single metal test cylinder lined with the treated netting. Twenty mosquitoes are introduced into the test chamber and the number of knocked-down and still mobile test mosquitoes is documented after 1 minute. Afterward, all mosquitoes are transferred to treatment-free holding cages to follow the 24-hour mortality rate.

The HTSS was used in a screening process to identify new chemicals that could be cost-effective substitutes for DDT. Grieco et al.[18] compared the outcome of laboratory assays with field studies including release and recapture experiments in treated and untreated huts. Results from both approaches demonstrated the strong spatial-repellent potential of DDT, followed by its contact-irritant properties, and finally toxic action. The HTSS was also used to learn more about the effects of standard vector control compounds on the behavior of *Aedes aegypti*. Pyrethroids such as α-cypermethrin, deltamethrin, and permethrin elicited a great contact irritancy, but still caused high knockdown and mortality rates, whereas the action of dieldrin was toxic with no indications of contact-irritant or spatial-repellent properties.[59] Similar observations were made in trials with *Anopheles albimanus*. Significant contact-irritant responses were documented for pyrethroids (α-cypermethrin, deltamethrin, and permethrin), DDT, and propoxur, whereas spatial repellency was only observed in trials with DDT.[60]

All these studies show that commonly used insecticides have different impacts on mosquito behavior, which can exceed their role as a killing agent. Sublethal effects such as contact irritancy and spatial repellency can contribute to a reduction in human–vector contact and thereby play an important role in breaking disease transmission.[18,59,60]

Y-Tube Olfactometer

Recently, a WHO guideline[58] on test methods for spatial repellents was published, which complements protocols on testing insecticidal activities.[61] The new guideline addresses testing methods for airborne chemicals, which may elicit an oriented movement away from the source, interfere with host finding, or change feeding responses and thereby reduce host–vector contact.

The exposure to airborne chemicals does not always result in a steered motion in the opposite direction. Some chemicals impede the host-finding process and are therefore called *attraction*

inhibitors.[62] Such a feature is of particular interest as spatial repellents that interfere with the mosquitoes' ability to locate a host are promising candidates to be used in push–pull vector control strategies.[20,62,63]

Y-tube olfactometers (Figure 6.4) are generally used to measure the level of attraction or repulsion of host-seeking mosquitoes to volatile stimuli in choice experiments.[62,64–66] Clean and conditioned air constantly runs through the tube system to the end of the base leg, where mosquitoes are connected. During stimulus application, mosquitoes are allowed to fly upwind into a decision chamber to choose between a test cage that holds the test stimulus and a control cage with clean air.

In attraction-inhibition assays, deterring stimuli are presented in combination with attractive odors (either coming from a synthetic blend or human hand) to measure the reduction in attraction elicited by the deterrent.[20,63,67] The use of synthetic blends containing combinations of L-lactic acid, ammonia, hexanoic acid, or acetone[68–70] helps to create more standardized conditions by reducing the variability that is known for human odors. A synthetic attractant blend can closely approximate the natural host, but never replace it.

In our bioassays, human odors that are highly attractive to *Aedes aegypti* are used to evaluate the potential of spatial repellents in Y-tubes. Mosquitoes are preexposed to the test chemical, which is dropped onto a filter paper and held into the apparatus behind cage 2 or 3 (Figure 6.4). After 15 seconds, the positive stimulus (finger) is introduced into the same port and mosquitoes are liberated into the system. By comparing the proportion of mosquitoes that are attracted to the finger in the presence and absence of the test chemical, the level of inhibition can be determined through the decrease in the attraction observed. Chemicals with known contact-repellent properties such as picaridin should be included as a negative control. The use of picaridin as a negative control is based on observations made during repellent efficacy cage tests. At normally applied topical concentrations, *Aedes aegypti* tends to land on the treated skin but then immediately takes off again, indicating that repellency requires direct contact. In Y-tube olfactometer assays, picaridin did not reduce the test mosquitoes' attraction to the natural host odors. Results from spatial repellency assays using olfactometers are presented in the section "Spatial Repellency of Natural Compounds."

Olfactometer tests are a quick and efficient way to evaluate the behavioral responses of mosquitoes to volatile stimuli; however, they sometimes overestimate efficacy because of the restrictions related to a confined volume and short distances from the point source release of the odors. Thus, results from olfactometer assays may not correlate well with field results obtained with the same test

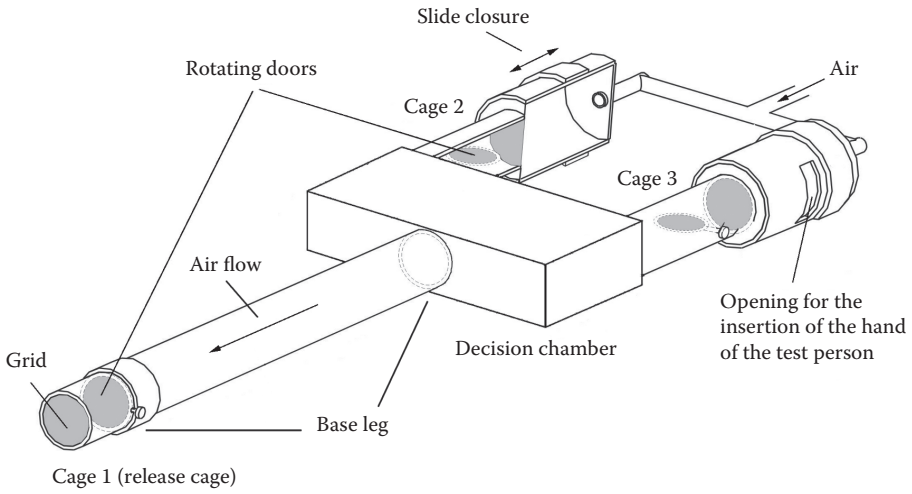

Figure 6.4 Y-tube olfactometer according to Geier and Boeckh. (From Geier, M., and J. Boeckh, *Entomologia Experimentalis et Applicata*, 92, 9, 1999.)

chemicals. These issues are addressed in a new experimental setup that includes a room test with a repellent-loaded air curtain that has to be overcome by the host-seeking test mosquitoes to reach the source of attraction (see the next section "Testing the Attraction Inhibition").

Free-Flight Rooms

Testing the Attraction Inhibition

A novel experimental setup was designed to investigate the spatial efficacy of chemicals on a larger and more realistic scale. Candidate materials are dispensed within a repellent-loaded air curtain in front of a tent opening (Figure 6.5). The dispensing system uses pressurized air, which is led through a wash bottle containing the candidate material. Consecutively, the repellent-enriched air is released through a perforated polyethylene (PE) tube at the top of the tent opening. Our experiments involved the BG-Sentinel™ (BGS) trap (Biogents AG, Regensburg, Germany) to attract *Aedes aegypti* to fly into the tent. The BGS is a superior trap for *Aedes* species, such as *A. albopictus*, *A. aegypti*, and *A. polynesiensis*, even without the use of CO_2.[71–74] The trap attracts host-seeking females by mimicking the convection currents produced by a human body; through visual cues; and by emitting artificial host odors from a synthetic dispenser, the BG-Mesh Lure. The synthetic lure is composed of lactic acid, hexanoic acid, and ammonia, compounds that are known to play an important role in the host-finding process of *A. aegypti*.[66] In the field, the BGS trap yielded greater catch rates compared to other collection devices such as the Fay–Prince trap or the Mosquito Magnet Liberty™ trap and was suggested to be used as a surrogate for human landing collections.[75]

Figure 6.5 Room test setup with repellent-dispensing system and BG-Sentinel trap. (From Obermayr et al., *J. Med. Entomol.,* 49 (6), 1387, 2012.)

The dispensing device is switched on and 10 test mosquitoes that have been preselected for host-seeking behavior are released into the room at the opposite side of the tent. To reach the positive stimulus, they must fly through the repellent-loaded air curtain. By comparing the number of mosquitoes that reached the tent in the presence and absence of deterring stimuli, the level of attraction inhibition can be estimated through a decrease in trap catches. A 5% blend of catnip oil and homopiperazine reduced BGS trap catches by an average of 60%, whereas using 5% catnip alone yielded an average reduction by 30%.[20]

When the trap is replaced by a human volunteer, the previously observed spatial effects are greatly diminished. In such trials, human landing rates usually reach 100%, indicating the strong attraction to the natural host. In contrast to olfactometer assays and room tests with the BGS trap, additional stimuli like CO_2, body heat, and visual cues from a human host greatly enhance attraction and may override the ability of the attraction inhibitors to block the perception of other host-produced kairomones. The discrepancy observed between Y-tube olfactometer and room tests with human volunteers also demonstrates that although olfactometer assays are suited to discriminate between spatial- and contact-repellent properties, they do not provide a reliable indication of the magnitude and quality of distance effects.

The free-flight room test setup can be modified to create a simple push–pull situation and evaluate the combinatory effects of BGS traps and spatial repellents to protect a volunteer inside the tent. This experimental approach is discussed in more detail in the section "Spatial Repellents and Their Use in Push–Pull Systems."

Testing the Nontoxic Actions of Formulated Products

The landing and feeding inhibition of formulated products can be assessed within one or two test rooms with cohorts of free-flying mosquitoes. Using these tests, the optimum effective dosage and duration of a protective effect can be determined. Formulated products are applied according to the label claim and mosquitoes are released into the same room or into an adjacent connecting room (when rates of mosquito room entry are measured). A volunteer records the level of mosquitoes landing and/or feeding in the presence and absence of the product; in this way, landing and/or feeding inhibition can be calculated according to the following formula:

$$\%\text{Inhibition} = \frac{(C - T)}{C}$$

where C is the number of mosquitoes landing or feeding in the control space and T the number of mosquitoes landing in the treatment space. The biological efficacy of a formulated product should yield a landing and/or feeding inhibition greater than 50%.[58]

SEMIFIELD TESTS IN SCREENED OUTDOOR CAGES

Screened outdoor cages with a volume of 300–815 m^3 have been used to simulate true environmental conditions, but offer the benefit of reduced variability in comparison to field tests.[13,62] Outdoor cages studies allow the use of a defined number of test mosquitoes at a certain age and physiological stage and can be performed with standardized arrangements of huts, traps, and release devices. WHO[58] recommends the use of semifield environments for the evaluation of formulated products following a protocol that is similar to the one for indoor testing (see the section "Testing the Nontoxic Actions of Formulated Products"). Semifield tests have been used to investigate the spatial potential of new materials and commercially available repellent products.[13,62,76]

The typical study design involves at least two identical cages, one for treatment and one for control trials, built in close proximity to each other. The efficacy of a formulated product is evaluated by measuring human landing and feeding rates in comparison with control trials. The test treatment is installed in the center of the cage, with the volunteer sitting on one end and the mosquitoes being released at the opposite end. Typically, 100 mosquitoes are liberated within one trial and human landing collections are performed by one volunteer. Mean mosquito collection rates during treatment trials are compared to controls. To ensure adequate host-seeking activity, the landing and feeding response in control trials should be greater than 50% and 25%, respectively. The level of landing and feeding inhibition in test trials is then calculated according to the formula shown in the "Testing the Nontoxic Actions of Formulated Products."

Insecticides producing high-vapor-phase concentrations have been evaluated in semifield situations.[13] Hessian strips, woven fabric made from natural sisal fibers, at a size of 4.0 m × 0.3 m were treated with 10 mL of transfluthrin and attached to 4 wooden poles surrounding a volunteer in the center of a screened cage. A total of 50 laboratory-reared and host-seeking *Anopheles arabiensis* females were used in each trial, and human landing catches were conducted for 2 hours per night. Over a testing period of 6 months, the treated strips provided more than 90% protection from bites.[13]

Kline suggested the use of outdoor cages to evaluate the attraction-inhibiting potential of spatial repellents (D. L. Kline, unpublished data). Attractive traps using synthetic blend dispensers are installed in the center of the cage, which is surrounded by 4 spatial-repellent releasing devices attached to poles at the corners of a 2.4 m × 2.4 m perimeter around the trap. The trap and release devices are switched on at least 30 minutes before mosquitoes are released into the cage, and trap catches are documented after a certain sampling period, for example, 12 hours. Afterward, human landing collections are performed at different locations outside the 2.4 m × 2.4 m perimeter to evaluate the extent of spatial repellency produced by the dispensed chemical.

Outdoor cages have also been used to document trap catch rates in the presence of the repellent-dispensing device described in the section "Testing the Attraction Inhibition" (unpublished data). Within this particular setup, a BGS trap fitted with a BG Lure dispenser is installed inside a tent located in the outdoor cage. The back and front openings of the tent are sealed with fine mosquito netting to allow air to circulate through the tent. The front net has a 1.2 m × 2 m cutout with the dispensing system installed at the top. The system uses outdoor air provided by a compressor, which is circulated through a container holding the test repellent and released through a perforated PE tube at a flow rate of 0.1 m/s. Mosquitoes have to fly through the repellent-loaded air curtain emitted by the dispensing device to reach the trap and get caught. One hundred laboratory-reared, host-seeking *Aedes aegypti* mosquitoes are released per trial, and the trap catch rate is documented after 1, 2, and 3 hours and compared to control trials without repellent. In alternative trials, the BGS trap is replaced by a human volunteer to compare human landing catches in the presence or absence of deterring stimuli.

FIELD TESTS USING EXPERIMENTAL HUTS

Early Setups to Evaluate the Toxicity and Irritancy of Dichlorodiphenyltrichloroethane

According to the WHO[77], an experimental hut is "a simulated house, in which all entering, exiting, dead, and blood-fed mosquitoes can be recorded." Malaria campaigns pushed the development and use of experimental huts to evaluate the efficiency of residual insecticides, and they are still in use today to assess the impact of IRS or insecticide-treated bednets on local vector populations.[77] The first experimental huts resembled typical African houses, but were standardized in shape, size, and furnishing to ease mosquito collections.[78,79] Initially, huts were used to study insecticide toxicity

and to determine optimum dosages to achieve high mortality.[34,43,80] As early field observations confirmed, the excitant and irritant effects of DDT[34,35,80] that had previously been reported in laboratory trials,[4] it became evident that the house-entering and -exiting behavior of vector mosquitoes needed to be examined in more detail. Hocking[34] studied the residual action of DDT on *Anopheles gambiae* and *A. funestus* and documented a reduction in their numbers inside huts or tents treated with different dosages of DDT (50, 100, and 200 mg/ft^2). He concluded that the lower mosquito abundance at treated sites was due to an increased rate of exiting as he did not observe any changes in the mosquito entering behavior. To prevent mosquitoes from escaping unharmed after having made contact with a treated wall surface, Hocking recommended the use of the highest tested dose of 200 mg DDT/ft^2 to ensure adequate mortality. In Uganda, different field sites were used to compare the house-entering behavior of *A. funestus*.[36] Overall, indoor mosquito collections were reduced by around 80% in treated huts and this was attributed to the contact-irritant effects of DDT. The reduction was most prominent in one hut that had been additionally treated outside on the ingress surfaces (doors, doorframes, and eaves). Apparently, the outside treatment contributed to preventing the mosquitoes from entering, an effect that was studied in more detail after Smith[81] introduced a new experimental hut type.

Traditional huts used window traps to collect exiting mosquitoes. Fine mosquito netting was attached to a wooden framework in a boxlike shape to be hung in front of the windows. Additional louvers and slits made it difficult for the mosquitoes to get out of the trap.[80,82] One disadvantage of the traditional hut design, however, was that it did not provide means to measure the number of mosquitoes that left through other egress ports such as the eaves. Smith[83,84] doubted that a reduction in indoor collections was due solely to a reduced house entry. He concluded that insecticides with excitant properties caused mosquitoes to leave the huts earlier during the night, at a time when window traps were less efficient due to the lack of light and exit was most likely to occur through the eaves. He used netting cages surrounding treated huts and additional eave traps to show that 33%–97% of the total egress indeed happened through the eaves.[83,84]

In 1965, Smith[82] introduced an improved hut type combining the advantages of a window trap system with an additional verandah trap that permitted the capture of mosquitoes escaping through the eaves. Between 1965 and 1969, the new verandah-type huts were used in extensive field studies in Kenya and Tanzania. In these studies, nominal doses of DDT (200 µg/cm^2) deterred 60%–70% of *A. gambiae* from entering the huts and drove 50%–70% of the blood-fed individuals outdoors after feeding. This deterrent effect lasted for at least 4 months after treatment.

In addition, gas chromatographic techniques were employed to assess the outflow of DDT from the huts and to follow the buildup of deposits on untreated sites. Mosquito deterrency was explained by a high initial outflow of DDT that reached 40 ng/cm^2/day. After 7 months, the outflow had reduced to 0.2 ng/cm^2, but deposits of 20–270 ng/cm^2 had built up around the eaves, causing deterrency at the ingress sites. Mosquitoes that left the hut were analyzed for the amount of DDT that they had picked up. Surviving mosquitoes had incorporated about 1.5 ng DDT, whereas dead mosquitoes showed amounts of 7–20 ng.[37]

Current Use of Experimental Huts

The WHO[77] recommends the use of experimental huts for small-scale field trials to measure the efficacy and residual activity of insecticides on wild mosquito populations. Several huts identical in construction should be used for treatment and control. Each hut receives one sleeper to stay overnight for a standardized period of time. Mosquito collections are conducted early in the morning from the room, the veranda, and exit traps. The number of alive and dead mosquitoes and fed and unfed females and their parity status (semigravid or gravid) are documented. Live mosquitoes are provided with 10% sugar solution, and their mortality rate is assessed at 24 hours.

Insecticide efficacy is evaluated by four indicators: (1) entry rate, representing the total number of mosquitoes inside the hut and exit traps; (2) exit rate, the proportion of females in the exit traps compared to the total number inside the hut and traps; (3) blood-feeding rate, the proportion of blood-fed females compared to the total number of mosquitoes; and (4) mortality rate, the proportion of dead females found in the hut and after 24 hours.[77] The potential of an insecticide is estimated by the *personal protective effect*, which is determined through the reduction in blood-fed individuals compared to control huts, and the *overall insecticidal effect*, which is calculated according to the following formula:

$$\frac{100 \times (D_t - D_C)}{E_C}$$

where D_t is the total number of mosquitoes dying in the treated hut, D_c the total number of dead mosquitoes in the control hut, and E_c the total number of mosquitoes that entered the control hut. Statistical analysis should use Poisson regression to analyze differences between predominant mosquito species, for example, the number of mosquitoes entering after the intervention, proportion of exiting mosquitoes, proportion of dead mosquitoes, and proportion of blood-fed females. Treatments can be compared using nonparametric Kruskal–Wallis test.

Experimental huts have been traditionally and predominantly used to study the behavior of malaria mosquitoes; collect data on their population densities and movement patterns; and, most of all, analyze their response to common public health pesticides.[51,81,85,86] A portable hut design was introduced by Achee et al.[86] to analyze the flight behavior of local mosquito populations in Belize, Central America. Data from a house survey conducted close to the study site were used to design the portable hut to ensure comparability to local homes. Wild-caught *Anopheles darlingi* females were marked and released at distances of 0, 400, and 800 m from the portable hut, and recapture was determined through human landing collections. The average recapture rate was 16.4%; the greatest proportion was caught after being released at 0 m (29%) and 11.6% was recaptured at a distance of 400 m, which went down to 5.8% at 800 m. Results indicate a tendency of *A. darlingi* to search for human habitations. Behavioral impacts of insecticides were not part of this study but were investigated earlier in the district of Toledo, Belize.[85] Pretreatment trials showed that the predominant vector species *A. vestitipennis* entered houses early at night (peak around 8:45 PM) and left early in the morning (peak around 2:45 AM). Subsequently, one hut was sprayed with DDT, one hut received deltamethrin treatment, and one hut remained untreated for control collections. The DDT had a significant and powerful repellent impact on *A. vestitipennis*, reducing their entry rate by 97% compared to prespray trials. The excitorepellent action of deltamethrin was less prominent compared to DDT; however, mosquito house entry was still reduced by 66% compared to unsprayed conditions. Deltamethrin also caused mosquitoes to leave the huts 5 hours earlier compared to pretreatment trials.

There is also growing interest in learning more about the behavioral responses of other mosquito vectors, and experimental huts have repeatedly been used to study *Aedes aegypti*.[87–89] An improved experimental hut to follow the moving patterns of *A. aegypti* in Thailand was introduced by Chareonviriyapap et al.[89] The new hut resembled indigenous Thai homes, but had some structural adjustments that improved longevity and mosquito collections, such as a raised platform, cement ant traps, and a walkway around the entire hut for easy access to interception traps. To study *A. aegypti* entrance behavior, 100 marked mosquito females were released at a distance of 10 m from the hut. Interception traps were located indoors to capture entering mosquitoes. Two volunteers stayed inside the hut to produce host cues and make regular trap collections. In exiting trials, 100 marked mosquito females were released indoors and interception traps were fixed to the exterior walls to collect exiting mosquitoes. One volunteer stayed inside to produce host cues. Between

31% and 49% of the released mosquitoes entered the huts between 06:00 AM and 06:00 PM, whereas 13%–23% left the huts in exit trials.

Experimental huts are currently being used in a proof-of-concept research effort to evaluate the efficacy of a push–pull control strategy for *Aedes aegypti*.[90] Laboratory trials have shown that *A. aegypti* rested on dark surfaces, even if they had been treated with insecticides. Treatment with DDT or α-cypermethrin caused a greater agitation but no shift to safer sites (for more information, see the section "Spatial Repellency of Pyrethroids"). These findings were incorporated into a field study in Thailand, using 75% surface area coverage (SAC) of dark material treated with regular field application rates (FARs) or lower amounts (half FAR). Marked mosquitoes were released inside treated huts, and their exiting behavior was monitored regularly. In addition, one volunteer stayed under a bednet inside the hut to produce host cues. The overall exiting behavior was low, with an average of 9.7 individuals leaving the control hut per day. Compared to control rates, an increase in mosquito exiting behavior was observed in huts treated with half FAR of α-cypermethrin, causing an average of 18.1 (46%) mosquitoes to exit per day.

Experimental hut studies have greatly contributed to our knowledge of mosquito behavior in response to residual insecticides. Compared to early versions, the design has steadily improved and advanced while reducing the overall bias in data collecting. Although they are costly to construct, experimental huts benefit from the fact that they can be brought to any field site and allow a certain level of standardization in size and shape, number of interception traps, number of persons staying inside the hut, and level of furnishing. Experimental huts will be an important tool for studying the effects of push–pull control strategies involving spatial repellents and traps (see the section "Spatial Repellents and their Use in Push–Pull Control Systems").

SPATIAL REPELLENCY

A spatial repellent is a chemical that deters mosquitoes at a distance[8] and inhibits their ability to locate a host.[9] Spatial repellency has been widely used to describe the action of coils, mats, and passive emanaters, which release vaporized chemicals that affect mosquitoes at a distance and cause knockdown, mortality, repellency, or inhibition of feeding.[12] Compounds that mask attractive host odors and thereby impact mosquito host-finding behavior were suggested to be defined as attraction inhibitors.[62]

SPATIAL REPELLENCY OF PYRETHROIDS

Pyrethroids target voltage-gated sodium channels in the nerve axons, and behavioral effects can be attributed to a disruption in the organization of the peripheral sensory system.[91] The spatial repellency of pyrethroids is believed to be caused by high knockdown activity and intrinsic sublethal effects, which disrupt the orientation to the natural host and/or inhibit feeding.[2,92–94]

There is growing evidence that repellents interact with odorants and odorant receptors (ORs), thereby interfering with the odorant-driven host-seeking process.[12,95–98] Bohbot et al.[96] tested the molecular effects of different insect repellents and one novel synthetic pyrethroid with known repellent properties on *Aedes aegypti* ORs. The pyrethroid inhibited the OR response to an attractant in a similar way to 3,8-*para*-menthane-diol or nepetalactone. Results indicated that repellent effects of pyrethroids may be due to a combination of sublethal neurotoxic excitement and interactions with the olfactory system.[12,97]

Pyrethroids with a high vapor pressure, such as metofluthrin, transfluthrin, and allethrin, evaporate faster at ambient temperatures, resulting in high-vapor-phase concentrations of active ingredients that can produce a barrier effect.[15,62] Evaporation rates are further enhanced in product

applications such as plug-in vaporizers, mosquito coils, and mats. However, heating is not necessarily required to vaporize the active ingredients in impregnated plastic resins and passive paper emanators, offering new and cost-saving ways of dispensing the active ingredients.

Metofluthrin (SumiOne, Eminence) was synthesized by Sumitomo Chemical Co., Ltd., Japan,[99] and has been extensively studied over the past decade. The most common way of dispensing the chemical is in the form of multilayered strips made from plastic or paper and burnable coils. Argueta et al.[100] compared the spatial efficacy of transfluthrin- and metofluthrin-impregnated paper strips in an outdoor setting in Japan. Metofluthrin provided high spatial repellency and yielded a 95%–100% reduction in *Aedes albopictus* trap catches for more than 6 weeks after treatment. Transfluthrin was less effective, reducing trap catches by 44%–86%, and spatial repellency failed after 5 weeks after treatment. Field tests of plastic strips impregnated with 5% metofluthrin yielded a significant decrease in *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles gambiae* house density indices in intervention areas, reaching a reduction of 70%–100% for up to 11 weeks after treatment.[15,101–103] Laboratory wind tunnel tests of metofluthrin-impregnated paper strips indicated that the presence of an airborne active ingredient not only reduced the proportion of landing *Aedes aegypti* but also inhibited those that succeeded in landing from feeding.[104] Recently, metofluthrin became commercially available as a spatial-repellent clip-on (OFF! Clip-On Mosquito Repellent). The device contains 31.1% active ingredient enclosed in a cartridge and a fan to dispense the chemical into the air. The clip-on was evaluated in a field study in Florida with six volunteers.[14] During a testing period of 3 hours, biting rates by *Aedes albopictus* and *Aedes taeniorhynchus* were reduced by 70%–79%.

Revay et al.[105] compared the bite-reducing effects of seven commercially available mosquito control devices on field populations in Israel. Three spatial repellent products; two allethrin lanterns (ThermaCELL, OFF! PowerPad Lamp); and a fan device dispensing a blend of lemongrass, cinnamon, peppermint, and geranium oils (Terminix ALLCLEAR Tabletop Mosquito Repeller) were found to be significantly more effective in reducing human landing rates close to a volunteer than commercially available mosquito traps that used attracting cues such as light, scent, and CO_2 (Dynatrap®, Vortex® Electronic Insect Trap, and Blue Rhino® SV 3100).

A study in Jacksonville, Florida, compared the spatial efficacy of two pyrethroid-based dispenser products (ThermaCELL [allethrin] and OFF! Clip-On [metofluthrin]) with two products using natural oils (Lentek Bite Shield [geraniol] and Bug Button Mosquito Eliminater [essential oils]).[106] The spatial efficacy of the test devices was estimated by measuring *Aedes albopictus* trap catch rates. A total of five BGS traps were installed at the test site, four were equipped with the test devices, and one was used to monitor control catch rates. The commercially available allethrin and metofluthrin emanators were significantly more effective in reducing trap catch rates than the devices using natural oils. Compared to control rates, ThermaCELL and OFF! Clip-On provided a reduction of 76% and 64%, respectively. Traps that were equipped with Lentek Bite Shield and Bug Button yielded catch rates that were reduced by 43% and 17%, respectively.

Effective dosages of insecticides that elicit spatial repellency rather than toxic responses were investigated in initial field studies in Thailand and Vietnam. In one study, experimental huts were treated with DDT (2 g/m^2) or metofluthrin coils (0.00625%) and mosquito entry was documented in mark–release–recapture experiments involving laboratory colonies of *Aedes aegypti*.[11] Sentinel mosquito cohorts inside treated and untreated huts were used to follow knockdown and mortality. Air samples were taken at different locations inside and outside the huts and analyzed via gas chromatography mass spectrometry. The DDT did not cause knockdown or mortality in the sentinel mosquitoes but reduced *A. aegypti* hut entry by 53%–70%. Results from air sampling indicated indoor DDT concentrations between 0.74 and 1.42 µg/m^3. Metofluthrin coils reduced *A. aegypti* house entry by 58% and resulted in minimal knockdown and mortality in sentinel mosquitoes. Air samples taken close to the sentinel cohorts revealed metofluthrin concentrations between 0.001 and 0.11 µg/m^3 indoors and between 0.001 and 0.03 µg/m^3 outdoors.

When plastic strips impregnated with 5% metofluthrin were evaluated for their spatial activity against *A. aegypti* in Vietnam, indoor air concentration ranged between 0.16 and 0.43 μg/m^3. The strips yielded a 58%–68% reduction in mosquito house entry for up to 4 weeks after treatment.[103]

Chemicals that have historically been used in vector control programs, such as DDT, α-cypermethrin, and deltamethrin, are known to elicit contact-irritant and spatial-repellent responses, not only at regular FARs but also at lower levels.[90,107] A laboratory box assay was used to analyze *A. aegypti* resting behavior, including dark and light materials at different SACs. In both chemical-free and treated assays, mosquitoes preferred to rest on darker sites, even when they had been treated and they covered only a small area (25% dark:75% light). Treatment did not cause the mosquitoes to move to safer untreated sites but resulted in an intensified agitation elicited through contact irritancy.[90] These findings open up new possibilities for future developments in vector control tools.[10,11,90] Focal application of lower amounts of insecticides not only reduces costs but also limits the development of insecticide resistance by reducing selection pressure based on contact-mediated toxicity. Most important, the vector-modifying behavior provided by contact irritancy and spatial repellency contributes to a reduction in human–vector encounters, thereby lowering the risk of disease transmission.[10,11,90,107]

Spatial Repellency of Natural Compounds

Plant-derived materials have been used for centuries to repel biting arthropods, for example, by hanging bruised plant parts in houses, burning plant materials, or applying essential oils to the skin.[108] Recently, outdoor plantations of repellent plants such as wild sage, neem, lemongrass, and West Indian lantana were studied for their effect on mosquito house entry in rural tropical areas.[109] When *Lantana camara* was planted outdoors, up to 83% fewer *Anopheles funestus* were collected indoors compared to control houses.

There is growing interest in using botanical compounds as alternatives to synthetic chemicals. Plant sesquiterpenes are especially active against mosquitoes and other pests.[110] Twelve sesquiterpenes that share structural similarities and represent a range of mosquito-repellent activities were evaluated for spatial and contact repellency against *Aedes aegypti.* Based on the results, quantitative structure–activity relationship models were developed to identify key properties of the sesquiterpenes that can be used to predict spatial- and contact-repellent actions.[111]

Over the past decade, increased efforts have been directed toward the discovery and analysis of noninsecticidal spatial repellents and a few promising substances with such properties have been discovered, such as catnip and linalool. Kline et al.[63] observed the spatial effects of linalool, a volatile compound contained in a variety of essential oils. In combination with CO_2 and octenol-baited traps, linalool provided up to 50% reduction in mosquito collection rates. In triple-cage olfactometer trials, linalool and dehydrolinalool exhibited spatial repellency against *Aedes aegypti*, causing a decrease in overall flight activity and reducing the ability to locate a human-derived attracting blend.

Linalool's spatial-repellent properties have been studied against wild mosquito populations in Israel.[112] The bite-reducing effects of 5% citronella, 5% linalool, and 5% geraniol candles were compared to negative controls (paraffin) in an indoor environment. Compared to paraffin, linalool and geraniol reduced human biting rates by 71% and 86%, respectively, whereas citronella had less pronounced effects and reduced biting rates by only 29%.

One of the most promising and extensively studied natural candidates is catnip, *Nepeta cataria*, a member of the mint family. Nepetalactone, the major component of the oil, was reported to be repellent to 13 different insect families,[113] cockroaches,[114] mosquitoes,[115] and stable flies.[116] Its spatial efficacy against *Aedes aegypti* has been evaluated in several different laboratory assays. Triple-cage olfactometer trials indicated that catnip was more effective in inhibiting *A. aegypti* attraction to a synthetic blend (lactic acid and acetone) or human odors than deet.[67] In Y-tube olfactometer

assays, 5% catnip oil provided more than 80% reduction in the test mosquitoes' attraction to a finger.[20] When the same concentration was evaluated in room tests using the dispensing system described in the section "Testing the Attraction Inhibition" and a BGS trap, *A. aegypti* catch rates were reduced by 50% compared to controls without a repellent.[20] Peterson and Coats[117] tested the effect of catnip oil and its nepetalactone isomers in a static air chamber. The setup consisted of a glass tube with a central opening for the introduction of the test mosquitoes and lids to cover the ends. Test compounds were dissolved in acetone and applied to filter paper disks. One end of the chamber received a treated disk, whereas the other end remained repellent free and was provided with a solvent-treated disk. The distribution of test mosquitoes inside the chamber was documented 15 minutes after they had been liberated. All test compounds showed significant spatial activity against *A. aegypti*. Catnip oil repelled up to 59% of the test mosquitoes from the treatment site, whereas the isomers deterred 56% (*E,Z*-nepetalactone) and 50% (*Z,E*-nepetalactone). When deet was applied to the filter paper, only 10% of the test mosquitoes avoided the treated site.

Contact irritancy and spatial repellency of catnip oil were also evaluated in excitorepellency test chambers (as described in the section "Excitorepellency Test Chamber") against field-collected *A. aegypti* and *Anopheles harrisoni*. In contact trials, escape rates from chambers treated with 5% catnip oil were high, 80% for *Aedes aegypti* and 60% for *Anopheles harrisoni*. In noncontact trials, 40% of the exposed *Aedes aegypti* mosquitoes escaped from the treatment chamber, whereas the escape rate of *Anopheles harrisoni* was 68%.[118]

Field data on the spatial repellency of catnip or other natural compounds are scarce. Chauhan et al.[19] suggested a field bioassay to evaluate spatial effects by monitoring trap catches in the presence and absence of different repellents. A standard miniature light trap (John W. Hock Co., Gainesville, Florida) supplemented with additional CO_2 was surrounded by a 4 m × 4 m horizontal frame, which held a total of 16 repellent receptacles (1.5 mL PE tubes, 4 per side). The spatial potential of cypermethrin, vetiver oil, catnip oil, deet, and *E,Z*-dihydronepetalactone was evaluated against local mosquito species in Beltsville, Maryland. Dihydronepetalactone is a minor component of nepetalactone-rich catnip oils and has been reported to be highly repellent to mosquitoes and blackflies.[119] In Chauhan's field assays, deet and *E,Z*-dihydronepetalactone were the only compounds that showed spatial effects and were able to reduce trap catch rates by 37% and 25%, respectively.

Catnip also showed great spatial activity against stable flies, *Stomoxys calcitrans*. More than 70% of the tested flies were repelled from the treatment port in olfactometer trials. Catnip's spatial efficacy was further evaluated in greenhouses where flies were released. In these trials, one half of a greenhouse received treatment (20% catnip oil on filter paper), whereas the other half received solvent only (hexane). *S. calcitrans*' movement patterns were documented every hour, and the atmospheric concentration of catnip was determined by solid-phase microextraction. After 4 hours, 50% of the flies were repelled from the treated site and the catnip atmospheric concentration had reached a level that was sixfold higher compared to the start of the tests. A slow-release formulation using 10% catnip oil in wax pellets showed promising but short-lived effects in the field. In the first 3 hours after application, the abundance of stable flies was reduced by more than 95% in the treated areas; however, the spatial effects soon dissipated. After 3 hours, the catnip atmospheric concentration was reduced by 50% compared to the start of the tests, which may explain the loss of the spatial-repellent impact.[116]

Spatial Repellents and Their Use in Push–Pull Control Systems

The idea of push–pull goes back to late 1980s, when Pyke et al.[120] presented their control strategy for cotton moths that had become resistant to standard insecticides. Push–pull was suggested as a means of integrated pest management, an alternative approach to combat growing insecticide resistance by using non-toxic, sustainable, and cost-saving components to affect the abundance of an insect pest.

The establishment of push–pull strategies in vector control is a subject of great interest. The concept takes advantage of the fact that insects use a variety of semiochemicals to locate food sources, oviposition sites, or blood hosts.[121] Through the combinatory use of both deterring and attracting stimuli, the abundance of insect pests can be reduced in a given area by interfering with the ability of the target pests to find their preferred resource (push) and luring them to an alternative source where they are trapped and killed (pull). A strong spatial repellent that affects host-seeking mosquitoes at a distance is of great importance for such a strategy and crucial to the success of the system.

Sublethal doses of common insecticides have been discussed as push components for an *Aedes aegypti* control strategy.[10,11,90,107,122,123] Other studies have examined noninsecticidal spatial repellents, such as catnip,[20,67,116] linalool,[63] commercial repellents,[76] or outdoor plantations of mosquito-repelling plants.[109]

As discussed in the section "Spatial Repellency of Pyrethroids," sublethal doses of insecticides can deter mosquitoes away from their source of release. This deterrency, however, could also be elicited by neurotoxic effects causing mosquitoes to rest and seek shelter. Such behavioral reactions were observed during semifield tests by Kitau et al..[76] Outdoor screened cages were used to measure human landing rates and Mosquito Magnet trap catches in the presence and absence of commercially available repellent products. When a 15% deet-based repellent formulation was applied to the skin, *Anopheles gambiae* landing rates were reduced by 70%, whereas trap catches were increased by 50% compared to controls run without repellent. However, in trials with 0.1% allethrin coils mosquitoes were going for shelter and trap catches decreased compared to control trials, "rendering the 'pull' end ineffective."[76] The behavior-modifying effects of pyrethroids were thoroughly investigated in another study, by preexposing *Aedes aegypti* mosquitoes to three common insecticides, DDT, transfluthrin, and metofluthrin, and subsequently monitoring BGS trap catch rates in a semifield environment.[123] After having been exposed to standard doses of the chemicals for 6 hours, mosquitoes were introduced to the trapping setup immediately or with a delay of 12 hours. The DDT and metofluthrin had no impact on the recapture rate of *A. aegypti* compared to contact trials. In immediate trials, transfluthrin significantly reduced recapture rates, whereas delayed trials showed no significant changes in BGS trap catches. Results from both studies indicated that the success of a pull compound depended on the characteristics of the push compound.

Field data on push–pull mosquito control strategies are scarce, especially for botanically derived push compounds. The potential of catnip was studied in a push–pull setup in laboratory assays.[20] The room test described in the section "Testing the Attraction Inhibition" was modified to create a simple push and pull situation, with a volunteer sitting inside the tent and a BGS trap fitted with a BG Lure dispenser installed in front of the tent. Mosquitoes that were attracted by the human odors had to pass a repellent-loaded air curtain to reach the volunteer. In control experiments without spatial repellent, 10% of the test mosquitoes were caught by the BGS trap, whereas 90% reached the volunteer. When a 10% mix of catnip and homopiperazine was dispensed, trap catch rates reached 43%, whereas human landing collections went down to 52%. Our findings indicate that a push and pull system based on a combination of BGS trap and spatial repellents is capable of reducing human–vector contact within a confined area.

The use of the BGS trap as a pull component in *A. aegypti* vector control was also suggested by Salazar et al..[124] The group evaluated the recapture rates of varying numbers of BGS traps in screened outdoor cages in Thailand. Batches of 10, 25, 50, 100, 150, 200, or 250 mosquitoes were released per experiment, and trap catch rates were documented in regular time intervals after release. The majority of the released mosquitoes was recaptured within the first 4 hours of the experiment with BGS catch rates ranging between 66% (use of one trap) and 79% (use of four traps).

The success of a push–pull system for vector control relies on a strong spatial repellent that affects host-seeking mosquitoes in a way that they are deterred from their preferred host but are still attracted to alternative target traps. There are indications that some commonly used insecticides, such as allethrin and transfluthrin, do interfere with host seeking and cause the mosquitoes to seek

shelter, thereby reducing the effectiveness of the attractant trap. The BGS trap has been shown to be an effective pull component with noninsecticidal chemicals. However, more research needs to be done on its effectiveness in a control system with insecticidal spatial repellents as the push element. The promising effects observed with natural compounds in laboratory studies need to be evaluated in a field or semifield environment. These tests should also include different modes of delivering the spatial repellents and optimizing trap placement and trap numbers.

CONCLUSION

The phenomenon of excitorepellency has been extensively studied over the past seven decades. The range of methods available today allows us to highlight almost any aspect of insecticide–mosquito interaction: laboratory systems help us to understand the different impacts of new and known chemicals on mosquito behavior, field trials provide valuable insight into the real-world situations, and modern air-sampling techniques give us the opportunity to estimate doses that repel but do not kill the target vector. Sublethal doses could play an important role in new control approaches such as push and pull systems. To achieve success in a push–pull system, repellent compounds will be required that do not paralyze the mosquito but allow it to seek alternative attractant sources, resulting in increased trap catches and decreased human–vector contact. Although we have gained great insight into the behavioral reactions of mosquitoes to insecticides, we still need to learn more about the physiological basis of repellency caused by a toxicant. What influences whether a mosquito will seek shelter or remain attracted to a host? What other mechanisms might be involved in causing an avoidance reaction? Expanding our knowledge will broaden the spectrum of available application techniques and lead to the development of new and improved vector control strategies.

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CHAPTER 7

Evaluation of Repellent Efficacy in Reducing Disease Incidence

Sangoro P. Onyango and Sarah J. Moore

CONTENTS

INTRODUCTION

Repellents are currently used by millions of people worldwide to prevent nuisance bites from blood-feeding insects, and it is now a multi-million-dollar global industry.[1] Until recently, there was limited scientific evidence on the efficacy of repellents to reduce disease. However, several groups of animals, including passerine birds and white-faced capuchin monkeys, anoint themselves with leaves, fruit, and even millipedes that contain compounds that are proven deterrents of ticks and

mosquitoes.[2–3] This behavior is observed to increase at times when attacks from such arthropods are higher, as observed in capuchin monkeys of South and Central America.[4] This fascinating observation is an indication that the use of personal protection from blood-feeding arthropods must improve the biological fitness of the animal that applies such repellents by reducing energy expended on "host defensiveness" or reducing its susceptibility to arthropod-borne diseases.[5]

Although the inhabitants of tropical countries with low per capita incomes may still use smoke and plant materials to keep biting arthropods at bay, the majority of research into the highly effective mosquito repellents that are available today has been carried out by scientists employed by or funded by the military to protect troops stationed in high-disease-risk areas. Some of the world's most important programs involved in the understanding and prevention of arthropod-borne diseases have risen as a result of conflicts in tropical regions that lead to massive loss of life from diseases such as yellow fever, louse-borne typhus, and malaria.[6] Two of these discoveries, *N*,*N*-diethyl-3-methylbenzamide (deet), which is a topical repellent,[7] and long-lasting permethrin-treated clothing,[8] are reviewed in this chapter. Two other repellents are also reviewed: *p*-menthane-3,8-diol (PMD), a topical repellent discovered in China,[9] and mosquito coils that were developed by the private sector in Japan[10] are examples of area or spatial repellents (see the section "Mosquito Coils").

Topical repellents are oils or lotions applied to the exposed skin or clothes of the consumer, with the most safe and effective being deet, picaridin, and PMD. Picaridin will not be reviewed here, because there is, to date, no epidemiological evidence of its efficacy, although a well-designed trial to evaluate its efficacy against malaria is currently underway with results available in 2014.[11] Permethrin-treated clothing is impregnated with a safe pyrethroid insecticide and binding agent to allow the permethrin to adhere to the fabric even after several washes. Permethrin is a synthetic pyrethroid, which has been extensively tested by the military,[12–15] and is the only insecticide approved for this use category by the U.S. Environmental Protection Agency.[16] It is nonstaining, odorless, and resistant to ultraviolet light and safe for regular use as an excellent tool for long-term prevention of arthropod bites. Mosquito coils are spirally shaped coils made from organic fillers, binders, and additives that allow the organic components to smolder evenly and continuously, to which a volatile pyrethroid insecticide is added that evaporates as the coil smolders over several hours after it is ignited. They are classified as area (spatial) repellents. Spatial repellency is used here as a general term to refer to a range of insect behaviors induced by airborne chemicals that result in a reduction in human–vector contact. This can include knockdown, interference with host detection (attraction–inhibition), or movement away from a chemical stimulus.[17] Other forms of spatial repellents include vaporizers and mats that have available extensive phase II (laboratory) data demonstrating excellent efficacy[18] but no epidemiological evidence of efficacy to date.[19] Vaporizers and mats require electricity to evaporate the insecticide from a small liquid reservoir containing the insecticide and a cellulose mat impregnated with the insecticide, respectively. This feature limits their application for disease prevention in the rural tropics where the majority of vector-borne diseases occur, because electricity is not available. Another intervention of note is passive emanators that have a large surface area, allowing the passive diffusion of insecticides from the surface. There is extensive evidence from studies with dichlorvos that passive emanation of insecticides is effective against malaria vectors (Table 7.1). However, dichlorvos does not have a suitable toxicity profile for public health use.[20] The discovery of the extremely nontoxic pyrethroid insecticides metofluthrin and transfluthrin (reviewed in the section "Mosquito Coils") means that passive emanation of such compounds is an area of current research interest[21,22] and large-scale epidemiological trials regarding this topic will begin in the near future. This has been publicized on the Notre Dame website (http://news.nd.edu/news/46769-second-largest-research-award-at-notre-dame-fights-malaria-and-dengue-fever/). Development of such products will be of great value because although the pyrethroid insecticides used in coils are not harmful to humans, often the smoke produced from the combustion of coils is a nuisance to people, reducing consumer acceptance, and some brands generate products of incomplete combustion, which are harmful to humans.[23,24]

Table 7.1 Overview of Insect Vectors of Disease, Their Behavior, and Means of Preventing Bites

Vector	Disease	Location	Time of Biting	Indoors/ Outdoors	Transmission Season	Recommendation
Anopheles mosquitoes	Malaria	SSA	Dusk– to dawn with late night peak	Indoors and outdoors	All year with peak during and following the rainy season	Avoid mosquito bites especially after sunset by using insect repellents containing deet or PMD and long clothing impregnated with permethrin. Sleep beneath insecticide-impregnated bed nets. Sleep in air-conditioned/screened rooms where possible, and use mosquito coils containing transfluthrin, D-allethrin, or metofluthrin, if possible outdoors after dark
		SA	Dusk to dawn with early evening peak	Mainly outdoors	During and following the rainy season	
		CA		Indoors and outdoors		
		SEA				
		SCA				
	Lymphatic filariasis	Mainly SSA (75% of cases) some SCA, CA, SA—mainly in residents and long-term travelers >1month	Dusk to dawn with late night peak	Indoors and outdoors		
Aedes mosquitoes	Dengue fever	SSA	Daytime and early evening	Indoors and outdoors	All year round, but especially following the rainy season and during epidemics	Prevention of mosquito bites during daytime using a repellent with deet or PMD is essential during epidemics. Use of mosquito coils or heated mats indoors and sleeping in screened accommodation is advised
		SCA				
		SEA				
		CA				
		CAR				
		SA				

(*continued*)

Table 7.1 Overview of Insect Vectors of Disease, Their Behavior, and Means of Preventing Bites (*continued*)

Vector	Disease	Location	Time of Biting	Indoors/ Outdoors	Transmission Season	Recommendation
	Rift Valley fever	SSA, ME			During epidemics related to very high rainfall. chikungunya entering EU with climate change	
	Chikungunya	SSA, NAf & ME, SEA, EU				
	Yellow fever	SSA, SA			Can occur year round, but mainly during and following the rainy season and during epidemics	Ensure vaccination for yellow fever before traveling to endemic areas
Culex mosquitoes	Japanese encephalitis (JE)	SEA		Mainly outdoors	All year round, risk mainly among residents and travelers to rural areas	Ensure vaccination for JE before traveling to endemic areas. Avoid mosquito bites especially after sunset by using insect repellents containing deet or PMD and long clothing impregnated with permethrin. Sleep beneath insecticide-impregnated bed nets. Sleep in screened rooms where possible and use mosquito coils containing transfluthrin, d-allethrin, or metofluthrin if possible outdoors after dark
	Lymphatic filariasis	SSA, SCA, SEA, SA (Haiti, the Dominican Republic, Guyana, and Brazil)	Dusk to dawn with early evening peak	Indoors and outdoors	All year round, risk mainly among residents and long-term travelers	
	West Nile fever	SSA, NAf & ME, SCA, NA, EU			All year round in tropics, warmer months in northern hemisphere	

Sandflies	Leishmaniasis	SSA, SCA, CA, SA 90% of visceral leishmaniasis cases occur in India, Bangladesh, Nepal, Sudan, Ethiopia, and Brazil; 90% of cutaneous leishmaniasis cases occur in Afghanistan, Algeria, Iran, Saudi Arabia, Syria, Brazil, Bolivia, Colombia, and Peru	Most species are active at dawn and dusk and during the night, but in forests and dark rooms they may also attack in the daytime	Most species feed outdoors, but a few feed indoors	All year round	Use long clothing in areas where sandflies are common, as their short mouthparts cannot bite through clothes. Avoid sandfly bites, particularly after sunset, by using insect repellents containing deet or PMD and by wearing long clothing impregnated with permethrin. Sleep under insecticide-impregnated bed nets (small mesh) and in screened accommodation if possible
Blackflies	River blindness	Mainly SSA (West Africa) also CA, SA, mountainous wet areas, and southern Yemen	Daytime	Outdoors	All times of the year, but more common in residents and long-term travelers >3months	Avoid areas where blackflies are active—near large and fast-flowing rivers. Wear long, light-colored clothing treated with permethrin if habitat cannot be avoided
Deer flies	Loiasis	SSA (West and Central Africa) in rain-forested areas	Daytime	Outdoors	All times of the year but especially the rainy season and more common in residents and long-term travelers	Avoid areas where deer flies are active—near muddy rivers. Deer flies are attracted to wood smoke, so avoid campfires. Wear long clothing. Treat clothing with permethrin if habitat cannot be avoided
Biting midges	No disease, but severe nuisance	AU, NA, CA, EU Most northerly temperate regions	Crepuscular during dawn and dusk, but for most species biting activity peaks in the early evening. Biting in the daytime if conditions are humid, still, and cloudy	Outdoors	Spring, summer, and autumn when adults are present	Avoid areas where midges are active—breeding grounds are acid soils, boggy soils, or coastal salt marsh. Use repellents of choice. Wear midge hoods. Wear long, light-colored clothing and treat clothing with permethrin if habitat cannot be avoided

(*continued*)

Table 7.1 Overview of Insect Vectors of Disease, Their Behavior, and Means of Preventing Bites (*continued*)

Vector	Disease	Location	Time of Biting	Indoors/ Outdoors	Transmission Season	Recommendation
Tsetse flies	African sleeping sickness	SSA mainly Tanzania, Uganda, Malawi, and Zambia (East African form) Democratic Republic of Congo, Angola, Sudan, Central African Republic, Chad, and northern Uganda (West African form)	Daytime. East African tsetse prefer wooded thickets and west African tsetse are found in forests and vegetation along streams	Outdoors	All times of year	Avoid wearing dark blue or black clothing. Keep car windows closed when traveling through areas of woodland. Wear long permethrin-treated clothing if outdoors in tsetse habitats
Triatomine bugs	Chagas disease	CA and SA, mainly Bolivia	Night	Indoors in rural forested areas, especially in poor housing (mud walls and thatched roofs)	All times of year	Sleep under insecticide-impregnated bed nets. Move the bed away from the wall
Fleas	Plague	SSA, SCA, NA	Day or night	Indoors or outdoors	All times of year	Avoid areas of high rodent density (primary host). Wear a repellent containing deet and tuck trousers into socks to avoid bites around the ankles. Use an insecticide-treated bed net if sleeping in endemic areas
Hard ticks	Tick-borne encephalitis (TBE)	SCA, EU, SEA (China and Korea)	Day and night	Outdoors	Tropics: any time Temperate: spring and summer, although season extending due to climate change	Vaccine available in Europe and Canada, but not licensed for use in the United States Avoid areas where ticks are abundant in woody and bushy areas with high grass and leaf litter. Walk in the center of trails Examine clothes and skin for ticks regularly (at least daily), and remove them with forceps Wear long clothing and tuck clothing into boots Use repellents containing deet and permethrin on clothing

	Rickettsial diseases including spotted fevers and Q fever	SSA, SCA, SEA, NA, EU				
	Tularaemia Lyme borreliosis	SSA, NA, EU				
Soft ticks	Relapsing fever, borreliosis	SSA, SCA, SEA, NA, EU				
Chigger mites	Scrub typhus	SEA	Any time	Outdoors	All times of year	As for ticks

Note: SSA, Sub-Saharan Africa; NAf & ME, North Africa and Middle East; SCA, South Central Asia; SEA, Southeast Asia; AU, Australia; PI, Pacific Islands; NA, North America; CA, Central America; CAR, Caribbean; SA, South America; EU, Europe.

The annual market value of personal protection consumer products is over $2 billion for powders, gels, and repellents and $2.6 billion for spatial repellents including vaporizers and coils. It is estimated that 45–50 billion mosquito coils are used annually by approximately 2 billion people worldwide,[25] mainly in Southeast Asia, but with a growing market in South America and Africa. These products present a great opportunity for public health, because such products could provide a means of disease control that is already proved to be highly acceptable to end users, because those who can afford them are willing to buy them.

VECTOR BEHAVIOR MODIFICATION FOR DISEASE PREVENTION

The World Health Organization (WHO) has recommended that all travelers to disease-endemic areas should minimize exposure to insect bites by selecting a combination of personal protection methods including insect repellents, mosquito nets, mosquito coils, aerosol sprays, protective clothing, screening, and air-conditioning.[26] The U.S. Department of Defense spent $4 million in developing the insect repellent system that comprises the proper wearing of a permethrin-treated uniform, and the application of extended-duration deet lotion to exposed skin that, if used correctly, provides close to complete protection from arthropod-borne diseases.[27] However, there has been no discussion on the implementation of repellents for public health use. The main explanation behind this is that until recently there were insufficient studies conducted to convincingly demonstrate that repellents can be effective against disease transmission.

Public health vector control tools such as indoor residual spraying (IRS) and the use of long-lasting insecticide-treated nets (LLINs) are extremely effective in sub-Saharan Africa.[28] Massive mobilization of both financial and political resources of the past decade[29] has resulted in the scale-up of LLINs and IRS and has had a great impact on malaria transmission.[28] However, there is a substantial amount of disease transmission both within and outside of Africa,[30] where vector behavior evades control through conventional means such as insecticide-treated materials because vectors bite outdoors and at times when people are still active (Tables 7.2 and 7.3). Recent estimates are that 16% of global malaria burden and 8% of malaria mortality occur outside of Africa, whereas outbreaks of dengue and other arboviruses are increasing and spreading geographically.[31] Thus, tools targeting these outdoor and day biters are required. With the new impetus for malaria eradication of the past decade and the realization that the existing control tools LLINs and IRS cannot solely achieve this, repellents are increasingly being considered as the supplementary tool in appropriate scenarios.[32] Modern repellents are extremely effective in preventing human–vector contact. The burden of vector-borne disease remains elevated despite substantial gains in control. There remains a challenge to develop repellency as a vector control option to complement existing tools in scenarios where the vector[33] (Table 7.1) or the human population[32] (Table 7.2) exhibits behaviors that require their use.

How Repellents Work to Reduce Vectorial Capacity and Vector-Borne Disease

When considering vector control for disease prevention, it is useful to consider how repellents could reduce the vectorial capacity (VC) of the disease vector population of interest and thus reduce disease transmission. The concept of VC was derived from models of malaria transmission first devised by Ross and was developed to guide the first global malaria eradication plan.[34] VC is described by an equation (Box 7.1) and is defined as "the average number of inoculations with a specified parasite, originating from one case of malaria in unit time, that the population would distribute to man if all the vector females biting the case became infected."[35] The concept of VC is sufficiently simple that it can be applied with some modifications to account for varying vector behavior, competence, and ecology, as well as differences in the dynamics of infection, disease, and immunity in vertebrate hosts, and has been used to

Table 7.2 Human Behavior That Necessitate the Use of Complementary Control Tools

Activity	Region	Disease	Increased Risk of Disease	Vector	Reference	Prevention Strategy	Reference
Illegal gold mining	Amazon (Brazil)	Malaria	Prevalence of malaria among individuals involved in gold-mining activities (67%) OR = 1.92 (1.05–3.50), who came from nonendemic areas (43%) 1.56 (1.06–2.29), and who reported being outside after 5 PM (37%) 2.04 (1.06–3.95)	*Anopheles darlingi*	43, 44	Health education, provision of free repellents, and/or permethrin-treated clothing plus long-lasting insecticide-treated hammock nets to miners. Topical repellents in this region prevent 80% of malaria among users in the Bolivian Amazon OR = 0.20 (95% CI = 0.11–0.38) Permethrin-treated uniforms prevented malaria OR = 0.24 (95% CI = 0.07–0.87) among soldiers in Colombia—part of the Amazon region	45, 46
Open gold mining	Amazon (Bolivar state, Venezuela)	Malaria	Malaria was almost absent until the beginning of mining activities in the 1980s. Now, between 2001 and 2010, 72.3% (22,746 cases) are among men of working age mainly from mining camps	*Anopheles darlingi* and *Anopheles marajoara*	47, 48		
Overnight forest activities, e.g., hunting and travel	Amazon (French Guiana)	Malaria	3.3, 95% CI = 1.1–9.5	*Anopheles darlingi*	49		

(*continued*)

Table 7.2 Human Behavior That Necessitate the Use of Complementary Control Tools (*continued*)

Activity	Region	Disease	Increased Risk of Disease	Vector	Reference	Prevention Strategy	Reference
Agricultural expansion into forested areas	Amazon (Brazil)	Malaria	Possibly as a result of their more frequent involvement in forest-related high-risk activities, such as clearing land, males had a higher malaria incidence (30.7 [95% CI = 27.6–34.0] episodes per 100 person-years at risk) than females (21.4 [95% CI = 18.7–24.3] episodes per 100 person-years at risk), with a rate ratio of 1.39 (95% CI = 1.17–1.64, $p < .001$ by Fisher's exact test)	*Anopheles darlingi*	50, 51		
Migrant forest workers	Mekong (Thailand)	Malaria	Overnight stays in the forest carried a higher risk of malaria infection OR = 4.13 (95% CI = 1.29–13.13)		52		
Overnight forest activities, e.g., hunting and travel	Mekong (Lao PDR)	Malaria	Overnight stays in the forest carried a higher risk of malaria infection OR = 2.12 (95% CI = 1.14–3.95)	*Anopheles dirus*	53		
Collecting food in the forest bamboo, nuts, berries, game animals, and birds	Vietnam	Malaria	Forest work carried a higher risk of malaria infection OR = 2.86 (95% CI = 1.62–5.07) in men but not women OR = 0.71 (95% CI = 0.59–0.86)			Only 2.3% of the population used malaria prevention methods as they cannot afford them. Even after adjusting for the effect of forest work, ethnic group, age, and education, women were still significantly less at risk of malaria. Compared to men, women usually remain well covered, particularly when working outside, thus reducing the risk of exposure to mosquito bites	54

Rubber tapping	Mekong (Thailand)	Malaria	In an area where LLINs and IRS are applied, those earning daily income by performing labor activities mostly in agriculture such as rubber tapping and rubber sheet processing at the smallholdings of rubber plantations were at high risk of malaria OR = 2.92 (95% CI = 1.14–7.44)	*Anopheles dirus*, *Anopheles maculatus*, *Anopheles minimus*	55		
Orchards in tropical forested areas	Mekong (Thailand)	Malaria	Up to 30% acquired orchards planted on former forested areas	*Anopheles dirus*, *Anopheles minimus*	56	Use of personal protection such as repellents and permethrin-treated long clothing if working at dawn or dusk	
Organized gold and copper mining	Sumatra (Indonesia)	Malaria	90% Of imported malaria between 2009 and 2012 in Sukambumi health centers (West Java) was among miners from Sumatra who worked night shifts in mines		57, 58	Provision of permethrin-treated work wear by companies for those on night shift recommended. Use of permethrin-treated uniforms did not prevent malaria among soldiers in Thailand, although the design of the study may have influenced the results	59
Organized open pit gold mining	Iduapriem, Obuasi, Ghana; Siguiri, Guinea; Sadiola/ Yatela, Mali; and Geita, Tanzania	Malaria	2010 Malaria incidence per 100 employees Iduapriem 104.62, Obuasi 19.4, Siguiri 22.74, Sadiola/Yatela 9.04, Geita 6.68 despite US$2 million annual investment in control at the sites in LLINs, IRS, and health education	*Anopheles gambiae*, *Anopheles funestus*, *Anopheles arabiensis*	60, 61	Permethrin-treated work wear for night shift workers. Insecticide-treated clothing prevented malaria by 70% OR = 0.31 (95% CI not reported) in Kenya with *Anopheles gambiae, Anopheles funestus, Anopheles arabiensis* as the primary vectors	62

(*continued*)

Table 7.2 Human Behavior That Necessitate the Use of Complementary Control Tools (*continued*)

Activity	Region	Disease	Increased Risk of Disease	Vector	Reference	Prevention Strategy	Reference
Military	The Netherlands	Lyme disease		*Ixodes ricinus*		Use of protective clothing and boots reduced the risk of lyme disease in Dutch soldiers based outdoors to that of the control group based indoors	63
	New Guinea	Scrub typhus		*Trombicula* spp.		Field tests with dibutyl phthalate applied every 2 weeks to uniforms of Australian soldiers resulted in a 60% and 70% decrease in scrub typhus when it was given to two brigades	64
	South Pacific	Scrub typhus				Uniforms were sprayed with dimethyl phthalate or an emulsion formulation of dimethyl phthalate with an untreated control. All of the soldiers then performed combat operations for 7–10 days in areas with scrub typhus transmission. The dimethyl phthalate spray reduced the number of cases by 64% (from 45 cases in the control group to 16 cases in the sprayed group), and the emulsion reduced the number of cases by 94% (to 7 cases)	65
	Haiti	Dengue	16/241 Italian Army troops	*Stegomyia* (*Aedes*) *aegypti*		Skin repellents protective OR = 0.16 (95% CI = 0.05–0.56), permethrin-treated uniform protective OR = 0.35 (95% CI = 0.11–1.17)	66

			30/406 U.S. Army troops			Although 93 (93.0%) of all febrile patients reported insect bites, only 18 (18.2%) and 40 (40.4%) always used a topical insect repellent and a bed net, respectively. Few had used permethrin to treat the bed net (30.3%) or uniform (13.1%)	67
	Columbia	CL *Leishmania panamensis*	The greatest outbreak of CL occurred between 2005 and 2009, with more than 35,000 cases in the military forces, 80% caused by *Leishmania braziliensis* and 20% caused by *Leishmania panamensis*	*Lutzomyia. trapidoi, Lutzomyia gomezi, Lutzomyia panamensis, Lutzomyia yuilli*	68	The soldiers with treated uniforms exposed in an area with infected sandflies for 6.6 weeks had 83% less leishmaniasis (4 cases out of 143 soldiers) compared with soldiers with untreated uniforms (18 cases out of 143 soldiers)	46
	Egypt	Sandfly fever		*Phlebotamus papitasi*		The attack rate (probable immunes were disregarded from the data) among users was 2/77 and among controls was 9/83 = 0.24, which is a 76% reduction	69
	Sierra Leone	Malaria	93 Cases among deployed U.K. troops within 1 month	*Anopheles gambiae*	70	Insecticide-treated clothing was protective OR = 0.47 (95% CI = 0.20–1.05). Use of no personal protection increased the risk of malaria OR = 2.20 (95% CI = 0.79–6.17)	70
Religious gatherings	Venezuela	Malaria	Evangelic and Catholic revivalist sects gather outdoors every evening for hymn singing late into the night	*Anopheles Albitarsis, Anopheles oswaldoi, Anopheles nunetztovari, Anopheles triannulatus*	71	Ensure adequate prevention from mosquito bites using repellents and long clothing	

(*continued*)

Table 7.2 Human Behavior That Necessitate the Use of Complementary Control Tools (*continued*)

Activity	Region	Disease	Increased Risk of Disease	Vector	Reference	Prevention Strategy	Reference
Missionaries	Haiti	Dengue	After returning from a 1-week missionary trip to Haiti, DENV infection was confirmed in seven (25%). None practiced correct vector bite prevention strategies	*Stegomyia (Aedes) aegypti*	72		
Workers in the parks and forestry division	North America	Lyme disease	6.3% Seroprevalence among forestry workers and the odds of a recalled tick bite were five times higher among outdoor workers	*I. dammini*		Those who reported that they always used a repellent had a twofold lower seropositivity for lyme disease	73
	Poland	Lyme disease	In Poland in 2009, 664 /10,333 (6.4%) cases were certified as resulting from an occupational exposure among forest workers	*I. ricinus*	74	The use of permethrin-treated work wear reduces the probability of tick bites by 93%	75
Hiking	North America (Appalachian Trail)	Lyme disease	4% Of long-distance hikers contracted vector-borne disease—principally Lyme disease	Not mentioned, but most likely *I. dammini*	76	Subjects wearing treated summer-weight outfits (sneakers, socks, shorts, and T-shirts) were 3.36 times (OR = 3.36 with a 95% CI = [2.499, 4.526]) less likely to have nymphal *I. scapularis* attach to their body than subjects wearing untreated clothing. The odds of nymphal attachment, below the waist on the leg where ticks were applied to shoes, were 74 times less (OR = 73.60, 95% CI = [2.4, 551.45]) for the permethrin-treated group than the untreated group	77

Outdoor recreation—walking, camping, and hunting	North America (northwest California)	Lyme disease, human granulocytic ehrlichiosis	Number of nymphs attaching from sitting on logs: 1.44 per hour; gathering wood: 0.42 per hour, sitting against trees: 0.52 per hour, walking: 1.4 per hour, stirring and sitting on litter: 0.32 per hour, sitting on leaf litter: 0.24 per hour	*I. pacificus*	78

Table 7.3 Summary of Repellent Trials

Trial	Intervention	EPI Effect Size (OR)	VEC Effect Size	Primary Vector	Vector Feeding Behavior	Compliance	Other Points
Spatially Active Volatile Pyrethroids							
79	4 × 0.00975 Metofluthrin coils per house per night	0.39 (0.24–0.62)	32.9% Reduction in mosquito landings by human landing catch (HLC)	*Anopheles sundaicus*	33% of biting before 10 PM[80]	Nightly	
81	2 × 0.03% Transfluthrin coils per house per night	0.22 (0.13–0.39)	88% Reduction in indoor mosquito densities by CDC light trap	*Anopheles sinensis*	47% of biting before 10 PM[82]	>90%	
Topical Repellents							
83	15% Deet lotion in addition to PermaNet 2.0 LLINs	0.94 (0.59–1.48)	98.9% Protection for 5 hours in field tests	*Anopheles dirus*, *Anopheles minimus*, and *Anopheles maculatus*	20%–50% of biting before 10 PM[84]	About 50%	
85	Buzz Off repellent plus PermaNet LLIN	1.16 (0.75–1.80)	>80% Effective against *Anopheles gambiae* for 8 hours in laboratory tests	*Anopheles arabiensis*	70% Before 10 PM [86]	Not measured	Repellent arm had more malaria to begin with. Effect size calculated by study accounting for imbalance was 0.57 (0.35–0.94), $p = .028$
45	30% PMD lotion in addition to 25 mg/m^2 deltamethrin-impregnated bed net	0.05 (0.01–0.20)	Repellent provided 97% protection from *Anopheles darlingi* for 4 hours[87]	*Anopheles darlingi*	48% of biting before 9 PM[88]	>90% (Per protocol analysis)	
89	Repellent lotion containing 20% deet and *thanaka* (*Limonia acidissima*)	0.72 (0.50–1.05)	Repellent provided 65% reduction in exposure to *Anopheles minimus* and 85% reduction in exposure to *Anopheles maculatus*[90]	*Anopheles minimus* and *Anopheles maculatus*	*Anopheles minimus* 22% and *Anopheles maculatus* 62% before bedtime	Compliance actively detected at 84.6%	

91	15% Deet lotion in addition to Olyset LLINs	0.89 (0.69–1.13)	Repellent prevented >80% bites from *Anopheles arabiensis* over 4 hours	*Anopheles arabiensis*	30% before 10 PM	>90% (per protocol analysis) but application not adequately measured	
92	20% Deet and 0.5% permethrin soap	0.42 (0.25–0.69)	*Anopheles stephensi* and *Anopheles culicifacies* density—repellent prevented 100% bites over the whole night	100% effective	80% of anopheline biting before midnight	Self-reported compliance >95%	
			Permethrin-Treated Clothing				
59	Treated uniform with 2 g permethrin per uniform once every 6 months	0.96 (0.71–1.29)	100% effective for 3 months, 84.45% effective up to 6 months	*Anopheles dirus*	Not measured	100% compliance although it is not known if the uniforms were worn "correctly"	
62	Clothing treated with 0.37% permethrin–retreated every 3 weeks	0.56 (0.36 –0.86)	41% reduction in blood-fed mosquitoes in users' houses and 41% increase in fed mosquitoes in nonusers' houses	*Anopheles arabiensis*	Not measured	Not mentioned (assume all clothes were treated)	Reported odds of malaria in treatment group is 0.314 $p = .0002$
93	1 g/m^2 permethrin-treated *chaddars*	0.55 (0.38–0.78)	Reduced feeding success of *Anopheles nigerrimus*, *Anopheles stephensi*, and *Anopheles subpictus* by 0%–60%	*Anopheles stephensi*	80% of anopheline biting before midnight	Not measured	
46	Treated uniform with 600–712 mg/m^2 permethrin	0.24 (0.07–0.87)	Not measured	*Anopheles darlingi*	Not measured	Not measured	

BOX 7.1 VECTORIAL CAPACITY

$$C = \frac{ma^2bp^n}{-\ln p}$$

C = new infections disseminated per person per day by each mosquito
m = number of mosquitoes per person
a = probability a vector feeds on a host /day i.e the proportion of females feeding on man divided by the duration of the gonotrophic cycle in days
ma = the number of bites/man/day
p = probability of daily vector survival
$1/-\ln p$ = duration of the vector's life in days once it has survived the intrinsic incubation period
n = duration of the extrinsic incubation period in days
b = proportion of sporozoite positive mosquitoes that are infectious

understand the transmission of other vector-borne diseases, including dengue,[36] bluetongue,[37] onchocerciasis,[38,39] bancroftian filariasis,[40,41] and schistsomiasis.[42] VC describes the potential intensity of transmission by mosquitoes as a function of the (1) human-biting rate, representing the incidence of biting contact between the mosquito and humans in terms of the number of bites per person per day and indicating the number of vector females that could become infected per case per day; (2) expectation of infected life, which is days of infective life per mosquito infected with the given parasite species; and (3) human-biting habit, which is bites on a person per day by an individual female mosquito,[35] all of which can be measured using standard field collection techniques.[94] This exceedingly elegant means of considering the process and impact of vector control on human–vector contact and mosquito survival has been verified with field data[35] and provides a convenient logical framework to consider the impact of new vector controls. The majority of work involving the VC equation has considered insecticides that reduce both numbers and life expectancy of mosquitoes and have an excellent impact on reducing malaria intensity. However, in the original article in which VC was described, the author showed that by reducing the human-biting rate by 50% there was a consequent 75% reduction in the VC of the mosquito population.[35] VC is extremely sensitive to changes in the biting rate because a vector needs to bite twice to obtain and then transmit a pathogen—hence, human biting is squared in the equation (Ma^2). Thus, the use of repellents will have a strong effect on overall VC by reducing the probability of infecting or being infected by a vector, as described by Ma^2. Thus, when considering disease control we will define repellents as those interventions that reduce human–vector contact without killing a large proportion of the vector population, that is, those interventions that keep the human population and the vector population apart.

RANDOMIZED CONTROLLED TRIALS FOR MEASURING THE DISEASE IMPACT OF REPELLENTS

Different kinds of evaluations have been conducted to determine the effect of repellents on disease incidence. Randomized controlled trials (RCTs) are currently considered to be the gold standard for testing the effectiveness of interventions for disease reduction in a population,[95] provided that they are well conducted.[96] The most important feature of an RCT is that the individuals recruited into the trial are randomly assigned to the intervention or a control, thereby minimizing selection and allocation bias to control as much as possible for both known and unknown confounders that

could influence the correct measurement of impact of the intervention.[97] Other advantages of a well-conducted RCT are that it facilitates blinding of treatments from investigators, participants, and assessors to prevent bias in the estimation of intervention effect.[98] It allows for the use of probability theory that any difference seen between the different arms outside the treatment effect is due to chance. A large body of guidance is available to researchers on the importance of correct trial design,[99] implementation,[100,101] and reporting.[102]

The main disadvantage of RCTs is the limitation of external validity, that is, the results of an RCT may not be applicable to the general population, due to differences in geographical location, characteristics of the patients recruited, trial procedures, and methods of measuring the outcomes in the trial. For this reason, it is advised that standard methods to ensure quality and reporting guidelines are followed that will allow systematic review and meta-analysis, which aims to collate and synthesize data from multiple studies that meet prespecified eligibility criteria using methods that attempt to minimize bias.[99] The other disadvantages are cost and time. RCTs are quite expensive[103] and take several years until the results are published; thus, they may be less relevant at the time of publication.[104] However, when considering the public health implementation of a new vector control product the investment in an RCT is small when considering the importance of implementing a proven intervention that will save lives rather than wasting money on implementing an ineffective intervention (Christian Lengeler, pers. comm.). The cost of the series of RCTs used to generate evidence that bed nets prevented malaria[105] was less than $10 million; but between 2004 and 2010, $17 billion was spent on bed nets.[106]

Randomized Controlled Trials of Topical Repellents

Southeast Asia

In a refugee settlement in Pakistan, a household randomized trial of Mosbar (a soap containing 20% deet and 0.5% permethrin, which was lathered on but not rinsed off) versus a placebo lotion demonstrated a 56% reduction in *P. falciparum* malaria with an odds ratio (OR) of 0.44 (95% confidence interval [CI] = 0.25–0.76, p = .004) and a nonsignificant effect on *P. vivax* malaria with an OR of 1.29 (95% CI = 0.86–1.94, p = .226).[92] The study was carried out on a waterlogged land endemic for malaria, and transmission was effected by *Anopheles culicifacies*, *Anopheles stephensi*, *Anopheles nigerrimus*, and *Anopheles pulcherrimus*, which are predominantly early evening biting vectors.[92] This characteristic makes topical repellent use ideal as it is applied in the early evening, coinciding with the peak activity of these vectors. This local vector bionomic may have meant that the repellent reduced a substantial amount of malaria transmission and demonstrated the importance of studying the local vector bionomics to determine if the proposed intervention will have any impact on the vector population. The study used simple randomization to allocate treatment to the participants. Randomization minimized the allocation bias of the treatments and confounding factors that were not taken into account. Passive case detection of malaria cases was used, which might have led to the loss of cases that were not reported to the health clinic. Compliance was established by self-reporting of use every fortnight and therefore could not be conclusively ascertained. Field staff, laboratory technicians, and participants were blinded to the intervention. Although this study demonstrated an effect of repellents, it did not take into account the whole malaria transmission season. This study took place for only 6 months, during the *P. falciparum* transmission season and, therefore, demonstrated an effect only against *P. falciparum* malaria. No effect was shown against *P. vivax* malaria because the study was carried out when the transmission of *P. vivax* malaria was low and there were not enough cases to demonstrate a treatment effect. This study would have been stronger if it had been carried out longer to take into account both *P. falciparum* and *P. vivax* malaria transmission seasons. As *P. vivax* malaria is known to recrudesce, the study investigators should have cleared all malaria

cases through an appropriate treatment regimen after checking for individuals deficient in glucose-6-phosphate dehydrogenase (G6PD)[107] so that any cases that were observed would be classified as new malaria cases and not recurrent *P. vivax* cases. Thus, the investigators would have avoided losing malaria cases that they classified as recrudescent cases while they were actually new cases, which reduced the power of the study. It would also have been prudent if the investigators had used active case detection, where they visited all households recruited into the study and screened for malaria, instead of waiting for study participants to report to the camp's health facility. Thus, the investigators would have captured malaria cases of those individuals who visited alternative health facilities or chose to buy drugs directly from drugstores. Active case detection would have also allowed the inclusion of individuals who were too weak to visit the health facility for treatment or found the facility to be too far to seek services. Compliance could also have been better established by conducting frequent spot checks to determine if the study participants did indeed use the treatments they were issued.

In a refugee camp in Thailand, a double-blind randomized clinical trial on the effect of deet mixed with *thanaka* (a root paste made from pulp of the wood apple tree, *Limonia acidissima*, used locally as a cosmetic) compared to *thanaka* alone in pregnant women demonstrated a 28% reduction in malaria incidence, 10.6% (95% CI: 7.5%–13.5%) in women who used *thanaka* and deet, compared to the ones who used *thanaka* alone 14.8% (95% CI: 9.9%–19.7%) in *P. falciparum* malaria, although the difference was not statistically significant.[89] There was also no significant difference in the transmission of *P. vivax* malaria between the two treatment arms. The lack of a treatment effect was most likely because of malaria transmission being too low to demonstrate a treatment effect as a result of effective and timely diagnosis and treatment of malaria in the camp. As women who were parasitemic during the study were more likely to be anemic on admission than women who had no documented malaria, the authors concluded that they were probably infected before the start of the study, although randomization was performed correctly because anemia was similar between those allocated to treatment and those allocated to control. By treating all the malaria cases before the start of the study so that all cases seen were contracted during the study period, may have reduced prior infection status to bias results, although this would have required a larger sample size and longer study period to observe any treatment effect. The study used both active and passive case detections, which were well correlated. This demonstrates that among individuals with lower immunity to malaria and thus more likely to suffer symptoms, and where malaria screening and treatment is accessible, free passive case detection may be closely related to the actual malaria burden existing in the community and this method can be used as an effective malaria surveillance tool. However, under other conditions, for example, where there are nonsymptomatic malaria carriers or health care is of low quality or is costly to the user, this may not be case. The principal vectors in this area are *Anopheles maculatus* and *Anopheles minimus*, vectors that exhibit a tendency to bite in the early evenings.[90] This vector behavior demonstrates a circumstance in which repellent use is beneficial, and the fact that no treatment effect was observed suggests that the sample size used was too small to observe the treatment effect or that it may have been useful to use a higher concentration than 20% deet to increase the duration of nightly protection. However, the major finding of the study was that there was no difference in the proportion of congenital abnormalities following the use of deet between treatment and control arms. Also, no deet was detected in the umbilical cord of 46 of 50 samples that were analyzed and none of the 30 samples of urine analyzed were found to contain more deet than the acceptable levels of 0.1 μg/mL. This study reaffirms that deet is safe to use in the second and third trimesters of pregnancy.[89]

In another household randomized, double-blinded placebo-controlled trial recently conducted in Lao-PDR, to determine the effect of 15% deet lotion topical repellent in addition to use of PermaNet 2.0 LLINs on incidence of malaria did not demonstrate any intervention effect.[83] Field trials of 10%–20% deet that were carried out demonstrated a 94% protection against all mosquito

bites. The major malaria vectors in this region are the *Anopheles dirus* complex and *A. minimus*, which are both outdoor and early evening biting vectors in the area,[82] a characteristic that made the repellent an ideal tool for controlling malaria transmission in this setting. However, although the repellent was well received with over 90% of participants reporting that they liked using the lotions, compliance was still low with fewer than 60% of the participants using the lotions more than 90% of the time. Focus group discussions revealed that the assumption that local populations were protected from night biting if they were provided with LLINs was not always true. Adult men and children reported spending time outdoors at night hunting and fishing; they may have benefited from using a longer lasting repellent or even permethrin-treated clothing when engaging in nighttime outdoor activities. These behavioral factors, no doubt, increased bias and reduced the power of the study to detect an effect, if any. The treatment and placebo lotions both smelt and felt the same when applied on skin and were presented in identical bottles identifiable only by a three-digit numerical code. Households were randomized to the treatments by drawing straws labeled with the codes of either the repellent or the placebo lotion. Follow-up visits were done on random dates to ascertain compliance, and the field staff, data entry clerks, and participants were blinded. However, it may have been possible for the participants to distinguish between the two treatments because placebo users were more likely to experience mosquito bites. Treatments were administered at the household level and to no more than 25% of households in any one village. This minimized the chances of treatment contamination, through diversion of mosquitoes from repellent to placebo users, and confusion of treatments, if individuals in the same household were issued different treatments. This might have led to treatment contamination, which can occur through treatment nonadherence (not using the recommended intervention because of perceived lack of effect) and treatment crossover (receiving the intervention intended for the other group in a trial, e.g., repellent users might give or sell their repellent to a neighbor). Both of these scenarios are common in repellent trials and create bias, resulting in an underestimation or overestimation of the treatment effect in either arm of the study. In future trials, this shortcoming can be addressed by using clusters of participants that do not interact with each other, for example, use of villages that are far apart to minimize the chances of participants interacting with each other.

A study carried out in a forest fringe in India to determine the effect of 12% deet used in conjunction with insecticide-treated mosquito nets (ITNs) on malaria incidence demonstrated a threefold (OR = 3.63, 95% CI = 2.27–5.79, $p < .001$) and a fivefold (OR = 5.14, 95% CI = 2.78–9.78, $p < .001$) protective efficacy of the intervention in the first and second years of the study, respectively, when compared to the control arm.[108] This study demonstrated a substantial effect of the use of mosquito repellents and ITNs against malaria. The major malaria vectors in this area are *A. dirus*, *Anopheles philippinensis*, and *A. minimus* which are generally early evening biting vectors[109] where the repellents would protect against early evening biting which may explain why the repellents were additionally effective in reducing malaria among users of ITNs compared to ITN-only users. The ITNs may confer communal protection by reducing vector populations,[110] with additional protection from repellent use. This integrated vector management (IVM) using different tools (repellents and ITNs) would therefore have reduced vector populations and host parasite reservoirs by reducing human–vector contact, thereby lowering malaria transmission in the community. The study investigators collected baseline data on malaria incidence and vector bionomics before implementation of the intervention and were therefore able to establish the correct baseline incidence, reducing the chances of underpowering the study by using a smaller sample size. The study was also carried out for 2 years after 1 year of baseline data collection. This increased the sample size of the study, further minimizing the chances of underpowering the study. The study had several positive features: it used active case detection, minimizing the chances of missing malaria cases in the community and making the estimation of treatment effect more robust. The research team also conducted random sniff checks to

ascertain compliance of use of mosquito repellents and ITNs. Another aspect of this study that might have led to such a big treatment effect being observed was the promotion of interventions through information, education, and communication (IEC). For an intervention to be effective, it has to be acceptable by the community. Unlike other repellent studies, this study used IEC, which motivated the community to take up the intervention. This approach demonstrated that repellents can be an effective malaria control strategy if the community is well informed and educated and the intervention is made available. Another finding of significance of this study is the further reduction of malaria incidence in the second year compared to the first year. This demonstrates that continuous implementation of an effective IVM tool can have a great impact on malaria transmission. However, the major shortcoming of this study was the paucity of information on how the findings were analyzed. This omission makes the findings questionable and surprising that the article was published owing to the lack of information on even what method was used to analyze the data, the lack of data on slide positivity rates for the second and third years of the study, and the highly questionable reliance on a converse interpretation of the risk ratio that was presented in the publication. The authors should have provided (1) raw data on the number of cases per 100 man-years per cluster or positivity rates in the first and second years, (2) information on which model was used to analyze the findings, (3) the reason why this model was preferred over other models, (4) information on how the data were interpreted, and (5) information on how bias was accounted for to make the findings credible to readers without having to rely on the interpretation of the authors. The study as presented could not be used in a systematic review.

South America

A household-randomized, double-blind placebo-controlled clinical trial was conducted in Bolivia among the users of a freshly impregnated ITN (25 mg/m^2 deltamethrin) plus either the insect repellent (*Corymbia maculata citriodon*) with a PMD concentration of 30% (MASTA, United Kingdom) for the treatment group or 0.1% clove oil for the placebo group.[45] The study demonstrated an 80% reduction incidence rate ratio (IRR) (0.2) (95% CI = 0.11–0.38, $p < 0.001$) in *P. vivax* malaria. However, the effect on *P. falciparum* malaria was not significant most likely due to a lack of power as the number of *P. falciparum* cases was too low to demonstrate any treatment effect. This might be because of an unexpected round of fogging as explained by the authors, but they also offer the more likely explanation that the study took place when transmission of *P. falciparum* was low. Sequential randomization of households was used to allocate treatments, and both the participants and field staff were blinded. Both these attributes increased the robustness of the study, as there was minimal chance of selection bias by the field staff or the participants not using the placebo. The use of a clove oil repellent was useful in this circumstance as both PMD and clove oil have a strong odor, which would suggest to the users that both were active repellents. However, there was always the chance of the control group realizing that they were issued with the placebo as the trial went on and dropping out of the study, thereby reducing its power because of decreased sample size. The study took place for only 4 months, and thus the effect of repellent over the whole malaria transmission period could not be determined. If the study had been conducted for longer to take into account the whole transmission season, then a treatment effect is more likely to have been observed against *P. falciparum* malaria or even a larger, more robust estimate of treatment effect observed as the sample size would have been larger, consequently reducing sampling error and improving effect estimates. The major vector found in this region *Anopheles darling* has a peak biting time from 8 to 10 PM[88] and is strongly exophagic and exophillic;[111] therefore, it is recommended that repellents be used at this time as people are not under their LLINs. The PMD is extremely effective against even high densities of local malaria vectors and is likely to have provided users relief from high densities

of mosquitoes during the wet season.[87] Overall, the study demonstrated that the use of mosquito repellents in the early evening in conjunction with LLINs in regions of early evening vector biting did have an impact on malaria incidence, strengthening the case for employment of IVM in malaria control. The compliance of the study participants was reported to be very high, underlined by their preference for PMD measured by focus groups,[112] and this was confirmed by random sniff checks by the field staff. The large treatment effect was likely a combination of a well designed and implemented trial methodology conducted in an area where vector bionomics precluded control by other means and where the repellent was well complied with because it was both highly effective against mosquitoes, and cosmetically acceptable to the local population using it.

Sub-Saharan Africa

In a cluster RCT conducted in Ethiopia to determine the effect of Buzz Off repellent on malaria, the odds of contracting malaria was reduced by 43% (OR = 0.57, 95% CI = 0.35–0.94, $p = .028$) for the participants using repellents to supplement PermaNet 2.0 LLINs.[85] In this study, data were collected by three cross-sectional surveys during the 4-month study. It would have been more prudent for the study investigators to conduct the study throughout the year to take into account the whole malaria transmission season and during the wet and dry seasons. This would have produced a more realistic estimate of malaria in this region. It would also have increased the sample size of the study, thereby decreasing the chances of occurrence of a type II error. Also, some cases of malaria may have been omitted as data were collected for only part of the transmission season. The authors of this trial did not outline the active ingredient and amount present in the repellent. Information on how randomization was conducted was missing; although there was good similarity between socioeconomic variables between the treatment arms, randomization could not have been performed correctly because at baseline the two treatment groups were not similar in terms of malaria prevalence. There was twice as much malaria in the repellent arm of the trial, the control arm complied with and had more LLINs, and two of the eight clusters were sprayed with dichlorodiphenyltrichloroethane (to which arm of the study these were allocated is not stated) and this might have confounded the results of the trial. This resulted in the investigators altering the analysis plan of the study. When the authors followed the analysis plan, outlined in their protocol, there was no difference seen between the treatment arms. As a consequence, the authors changed their analysis, which might have altered the treatment effect observed because the data were not designed to be analyzed in this way.

A double-blind placebo-controlled cluster-randomized trial of 15% deet topical repellent carried out in southwest Tanzania demonstrated a nonsignificant protective effect of 27% reduction in household malaria rates from 91.17 cases per 1000 person-years (95% CI = 198.42–380.76) in the control arm to 65.37 cases per 1000 person-years (95% CI = 110.10–240.84) in the intervention arm ($p = .40$, $z = 0.84$) using the intention-to-treat analysis.[91] These findings were, however, not significant, possibly because the study was underpowered. The major vector is *Anopheles arabiensis*, which bites both indoors and outdoors from 6 pm to 6 am, and it was estimated that a repellent could reduce around 30% of exposure based on the average time to bed of 9 PM. Both semifield and field evaluations of the efficacy of 15% deet repellent demonstrated >90% protection for 4 hours against *A. arabiensis* mosquitoes. However, the effectiveness of an intervention is a component of both efficacy and acceptability by the community of that intervention. Therefore, to ensure effectiveness the study team conducted three rounds of social marketing of the repellent in the study area to encourage usage. This had positive results as usage was reported at 95%. However, despite all these checks that were put in place during project implementation a treatment effect was still not observed. This was mainly due to two reasons: first, the study team overestimated the baseline malaria incidence by extrapolating incidence from all-cause fever data and therefore estimated a

sample size smaller than what was needed to observe a treatment effect. Second, a drought that occurred during the study period lowered malaria transmission such that a treatment effect could not be observed. In future studies, it would be useful to conduct baseline malaria incidence studies to establish correct incidence estimates for sample size calculation. Compliance was determined by self-reporting, which was done at the end of every month when field-workers visited the households to issue new bottles of repellent/placebo. Therefore, compliance in between the visits could not be ascertained. However, random sniff checks were conducted and these spot checks determined that the participants did indeed use the treatments issued. It would, however, have been practical to conduct the checks every fortnight and compare them with self-reported compliance to establish a correlation between the two methods of determining compliance. Passive case detection of malaria by rapid diagnostic tests (RDTs) was used at the local dispensary where participants were offered free diagnosis and treatment. People did not believe the results of negative RDTs and some stopped attending the dispensary, preferring to self-medicate with antimalarial drugs or attend the other health facility in the village that used clinical diagnosis. Also, the health dispensary recruited into the trial may have been sufficiently far from the homes of some participants to prompt them to access alternative health facilities or go to a nearby drugstore. In the future, it would be useful to recruit all health facilities and drugstores in the study area to avoid loss of malaria cases and carry out active case detection. All these factors might have contributed to a reduction in malaria cases, lowering the sample size, thereby underpowering the study. The randomization of interventions and blinding was done as effectively as possible for this case by using treatment and placebo lotions in identical bottles identifiable only by a three-digit code. Even then, as time went by participants realized that they were issued a placebo because they were continuously being bitten. As a result, there was some treatment contamination where placebo users did not use their intervention and repellent users sold their repellents to their neighbors, lowering the power of observing a treatment effect. It was also suspected that study participants gave their identification cards to relatives and friends to benefit from free health care. This would also lead to treatment contamination, which could be overcome with the use of a fingerprint scanner or photographic identification to identify study participants.

A field clinical trial conducted in Isfahan, Iran, to determine the effectiveness of deet sticks against leishmaniasis in 430 students (50% male, 50% female) did not demonstrate any treatment effect.[113] The intervention was reported to be effective for 18–20 hours, and its minimum effective concentration was 55–77 μg/cm^2. Deet placebo was randomized to 330 individuals and placebo stick was randomized to 100 controls, and the treatment allocation code of sticks was revealed only at the end of the study. The children were followed up for 10 months. The efficacy of these sticks was evaluated in terms of the reduction in infection by leishmaniasis using relative risk (RR). Confusingly, in the results section of the study the investigators reported a different number of treatments and controls: out of 200 students who were protected using the placebo pen 2 students acquired leishmaniasis, and out of 230 students who were protected using the deet pen 8 students acquired leishmaniasis. Thus, the study cannot be accurately interpreted.

CASE-CONTROL STUDIES

Apart from RCTs, case-control studies have been conducted to evaluate the impact of repellents on disease. Case controls are observational studies of people with disease and a suitable control group of persons without disease, where a potential risk factor is examined by comparing the frequency of occurrence of the risk factor between these two groups.[114] A number of case-control studies have been conducted to determine the effects of repellents on malaria incidence.

In Afghanistan, a case-control study was conducted through social marketing of Mosbar, a repellent soap containing 20% deet and 0.5% permethrin.[115] Cases and controls were recruited through passive case detection at a local clinic. The combined use of Mosbar and ITNs demonstrated a 69% reduction in the odds of contracting malaria (OR = 0.31, 95% CI = 0.13–0.72, $p = .007$) compared to control (neither Mosbar nor ITN). The local mosquito vectors *Anopheles stephensi* and *Anopheles nigerrimus* bite shortly after dusk, and throughout the night, a characteristic that makes the repellent a suitable control tool for evening protection before LLINs can be used. The repellent selected was highly efficacious and gave 100% protection for the whole night, which might have promoted the observation of treatment effect. However, as a hospital-based case control this study was prone to selection bias and therefore could not be generalized to the rest of the population, as individuals attending the clinics recruited into the trial might have had different characteristics from individuals in the general population. There are a number of anecdotal case-control studies that were not specially designed to measure the effect of repellents as shown in this study but to identify risk factors among those with malaria.

In a case control study of risk factors among British travellers returning from the Gambia less use of repellents was associated with a greater risk of contracting malaria.[116] The use of repellents, applied either on the skin or on clothes, is a key strategy for bite avoidance recommended in travel medicine. This finding illustrates the importance of using repellents when traveling to malaria-endemic regions. Therefore, all individuals traveling to malaria-prone areas should be advised to use malaria control strategies to protect against malaria. Also, tourist destinations should provide information on the vectors that are present in these regions so that the tourist can be better advised and prepared on which tools to use. It also emphasizes the importance of having international guidelines for travelers visiting malaria-endemic regions to avoid importing malaria cases to their mother countries.

In Kilifi, Kenya, in a large (>1500 participants), well-designed case-control study the use of local repellents, mosquito coils, and insecticide sprays was significantly associated with protection from developing severe malaria after adjusting for confounders (OR = 0.57, 95% CI = 0.35–0.94, $p = .02$). The cases and the controls were chosen from the same area in the community. Consequently, the results could also not be generalized to the whole population as the individuals from this area of the community might be different from other members of the community. It would have been better to select more than one study area to make the findings more general to the population.[117] A study from Gambia that used a design almost identical to the study in Kenya showed an association with the use of coils in preventing severe malaria in a univariate analysis, but this effect disappeared on multiple logistic regression.[118]

The overall evidence generated by the aforementioned studies demonstrates that the use of repellents can be effective against malaria transmission if these interventions are used correctly and with sufficient frequency. In studies where an association cannot be established, it is usually because of poor study design. The following series of studies are inconclusive due to a number of factors including poor marching; poor attention to sample size; and poor measurement of compliance, which is the single most important factor in the effectiveness of any repellent.

In another case study in India, individuals who did not use repellents had nonstatistically significant lower odds of malaria, with an OR of 0.85 (95% CI = 0.57–1.28, $p = .41$), compared to those who used repellents. This finding is not consistent with other repellent trials and there are various factors that might have led to this conclusion, especially as those exposed to higher levels of mosquito bites are more likely to use mosquito prevention tools. In addition, the cases and controls were not matched because the controls were recruited from the same clinic, assumed to have come from the same socioeconomic, demographic, and geographical area as the cases. Because of the study design, there was no way to establish compliance to repellent use. Also, the longevity and quality of these repellents could not be established, although the mosquito coils and mats used were reported to be allethrins and the topical repellents used contained diethyltoluamide, for which the concentration was not mentioned. The bionomics of the local vectors was not discussed to determine whether the use of repellents would be an appropriate tool.[119]

Similarly, in another case-control study in Burkina Faso use of mosquito coils and burning of plant leaves for smoke (spatial repellents) were not associated with a lower risk of malaria, with an OR of 1.24 (95% CI = 0.73–2.00, $p = .47$) and an OR of 0.74 (95% CI = 0.35– 1.56, $p = .43$), respectively. Like the aforementioned study, use of mosquito coils and burning of plant leaves for smoke were self-reported. The study participants might have overreported or underreported, biasing the findings on the study. The controls were recruited from the same residential area. As a result, these findings cannot be generalized to the whole population, as the individuals from this area might not have similar characteristics to the general population. The controls were not actively tested for malaria and were assumed to be malaria negative. This might have biased the study toward the null hypothesis if the controls were positive for malaria.[120]

In Ecuador and Peru, a community-randomized trial of Mosbar, a mosquito-repellent soap containing 20% deet and 0.5% permethrin, did not show any significant reduction in malaria incidence between the intervention and control groups.[121] The effect of the repellent soap was studied under different settings. It was found to be efficacious only when individuals wearing the soap were inactive after application. This contrasts with the findings from Pakistan[92] where the repellent was extremely effective in preventing mosquito bites. The differences observed might be due to the higher relative humidity in the Ecuadorian site that caused more rapid loss of repellent through sweating. Compliance to repellent use was not established and lack of treatment effect may have been due to poor compliance, as many people did not like the smell of the repellent and in Ecuador, because of humidity, a thick layer of soap remained on the skin, which was not pleasing to the users. As compliance requires a high degree of motivation, it was necessary for the study team to socially market their intervention to encourage its use and user acceptability. Interestingly, user compliance was drastically reduced when the soap was only made available from shops and was no longer available free of charge. This was similar to findings in other studies and underscores the importance of developing low-cost or highly subsidized interventions that can be accessed by those of low socioeconomic status in disease-endemic countries who are also those most at risk from disease morbidity and mortality. For an intervention to be effective, it has to be acceptable, affordable, or free to the community.

CROSS-SECTIONAL STUDIES

Cross-sectional studies are research methods that involve observing all of a population or a representative subset at a specific point in time. They collect data on outcomes and/or exposures collected on each participant at one moment in time. Thus, although they are simple and quick to perform, they are more robust at measuring associations with chronic diseases because they measure prevalent rather than incident outcomes. Cross-sectional studies that collect data on both outcome and exposure are not very robust in establishing the causal effect of an intervention, as they are prone to bias from confounding factors, but they can be used to test hypotheses about interventions and to justify a research objective.

A cross-sectional survey was carried out in the Thailand–Myanmar border in Northern Thailand to determine the risk factors that contribute to malaria infection. Malaria prevalence was extremely high in 46% of the participants. It was a well-designed study that had correctly used sample size calculation and demonstrated a clear relationship between working or staying overnight in the forest and having malaria in univariate and multivariate analyses, although the use of topical repellents and long clothing was protective against contracting malaria on univariate analysis, but this treatment effect was not seen when confounders were taken into account. This study shows some of the practical scenarios where topical repellents can be used, like individuals working in the forest or in crop fields who are not able to use conventional control measures like LLINs.[128]

A cross-sectional survey to determine the effect of personal protective measures (PPMs) against malaria in travelers demonstrated a significant reduction in malaria among travelers who used protective clothing covering their arms and legs. However, no significant reduction was associated with the use of repellents and coils. As explained in this study, compliance to PPMs was very poor among a large proportion of the study participants. This would likely explain the lack of treatment effect. Also, it is advisable that more stringent measures by responsible agencies are introduced to ensure compliance to PPMs by people traveling to malaria-endemic regions to avoid the exposure of nonimmune individuals to malaria and also reduce the importing of cases to their mother countries.[123] Compliance to personal protection is surprisingly low among those with access to the correct preventive measures. A recent survey among 2205 individuals from the French military during and after a stay in malaria-endemic areas were exposed to malaria incidence of 2.98 cases per 100 subject-years in malaria-endemic areas.[124] The "correct" compliance rates were 48.6% (95% CI: 46.5%–50.7%; ranging from 2.6% to 88.2%), 50.6% (95% CI: 48.5%–52.7%; ranging from 1.7% to 97.3%), and 18.5% (95% CI: 16.8%–20.1%; ranging from 4.9% to 59.6%) for wearing long clothing at night, using LLINs while sleeping, and using insect repellents, respectively. Factors that often influence compliance are gender, the rainy season, mosquito bite burden, and perceived mosquito attractiveness compared with other people, while perception of the severity of malaria was not associated with regular use of any of the methods measured. A further cross-sectional survey of 89,617 travelers returning from East Africa was conducted between 1988 and 1991.[123] Only 2% of respondents stated that they regularly complied with air-conditioned rooms and/or bed nets, adequate clothing, and use of insecticides and/or coils. Regular use of personal protection resulted in a small but significant reduction in malaria incidence when travelers were interviewed 12 weeks after returning home, but each method alone showed no significant effect. Unlike the situation among the French military travelers, the holidaymakers increased their compliance during periods when more mosquito bites were noticed; but similar to the French study, gender had no significant influence on compliance and, surprisingly, neither did diagnosed or suspected pregnancy. Those using no chemoprophylaxis were not more vigilant in preventing mosquito bites. Compliance diminished continuously with the length of stay in Africa: among those who stayed up to 2 weeks the compliance rate was 77.2%, whereas in those staying 2 months or more the rate was 63.3% ($p < .001$).

OUTBREAK REPORTS

In South Africa, topical application of 15% deet to feet and ankles reduced overall *Anopheles arabiensis* bites by 69% in field observations. This led to the testing of this intervention under operational conditions during a malaria outbreak in Mpumalanga, 15 km south of the Kruger National Park. The implementation of the intervention was associated with an immediate drop in malaria incidence from 42 to 10 cases per week. This effect is, however, difficult to interpret as it could have been due to repellent use and it could also have been due to the fact that the epidemic curve had peaked and was dropping naturally. The repellent may, however, have helped in maintaining the low incidence of malaria. But this study does give situations where repellents can be used. The most likely reason why the more effective LLINs were not used in this particular scenario is that the major vector in this area, *A. arabiensis*, had behaviorally adapted to outdoor biting and the secondary vector, *Anopheles funestus*, had developed resistance to IRS.[125] Although the results are not clear, this study represents a useful scenario in which repellents might be employed against malaria.

In an outbreak report that described the outbreak of *P. vivax* malaria in Far North Queensland, Australia, individuals who used topical repellents (deet) were at 0.01 (95% CI: 0.00–0.19) the odds of developing malaria compared to those not using repellents. The findings of this study reinforce the need to use other PPMs in areas when conventional malaria control tools are not applicable.[126]

During an outbreak in India, a well-designed investigation was conducted where malaria cases were slide-confirmed and compared with matched neighborhood controls. For both groups, information on personal protection use was gathered by questionnaires and data was compared using matched odds ratios (MORs).[127] In total, 7303 cases and 17 deaths were reported between April 2005 and March 2006 with a peak during the October rains (attack rate: 50 per 1000, case fatality: 0.2%), and half of the cases were detected by active case detection. Use of repellents was associated with an odds ratio of 0.1 (95% CI: 0.06–0.3) of contracting malaria, and failure to use repellents was associated with 69% of malaria cases in the population. Compared with controls, cases were more likely to sleep outdoors (MOR: 3.8, 95% CI: 2.2–6.5) and less likely to use mosquito nets and repellents (MOR: 0.3, 95% CI: 0.1–0.5). In this outbreak investigation, the villagers reported the use of repellents and coils and, therefore, correct and consistent compliance could not be established. This might have biased the treatment effect seen. Also, being a retrospective case control this study might have been prone to recall bias. Despite these shortcomings, this study demonstrated a protective trend of mosquito repellents against malaria.

There are a large number of disease outbreak reports among military personnel related to noncompliance with standard PPMs.[128] A report from the French Army monitoring leishmaniasis among troops stationed in Guinea showed four separate outbreaks of leishmaniasis in which the troops admitted that they did not use personal protection correctly.[129] In a malaria outbreak in French Guiana, a retrospective cohort study found that malaria was associated with a low compliance of impregnated battle dress uniforms (BDUs).[130] This study also shows the problem of compliance to repellent use. As studies mentioned earlier have shown, for repellents to be effective they must be acceptable to the individuals to whom they are issued and must be used correctly and consistently. Similarly, in a malaria outbreak in Sierra Leone among British soldiers a case-control study demonstrated that the use of insecticide-treated clothing offered significant protection against malaria with almost 50% fewer cases being reported among those individuals who used their impregnated BDUs (OR = 0.57, 95% CI = 0.20–1.05, $p = .045$). Interestingly, the use of multiple protection measures gave even better protection (OR = 0.29, 95% CI = 0.10–0.80, $p = 0.007$). However, the use of repellents and chemoprophylaxis showed no significant effect.[70] In a malaria outbreak in 2003, 44 U.S. Marines were evacuated from Liberia with either confirmed or presumed *P. falciparum* malaria.[131]An outbreak investigation showed that only 19 (45%) used insect repellents, 5 (12%) used permethrin-treated clothing, and none used bed netting, demonstrating further the importance of compliance in personal protection from vector-borne diseases.

PERMETHRIN-TREATED CLOTHING EVALUATION

Randomized Controlled Trials

Southern and Southeast Asia

In Afghanistan, an RCT on 1 g/m^2 permethrin-impregnated *chaddars* (cloth used as a head covering [and veil and shawl] by Muslim and Hindu women) reduced the odds of having *P. falciparum* and *P. vivax* malaria by 64%, OR = 0.36 (95% CI = 0.20, $p = .001$), and 38%, OR = 0.62 (95% CI = 0.36–1.06, $p = .069$), respectively. There was a significant effect in the 0- to 10-year and 10- to 20-year age groups. This trial, however, showed no effect on malaria incidence in refugees >20 years of age.[93] In this study, no information was given on how the randomization was carried out. The trial took place over 5 months and, therefore, did not capture the effect of repellents over the entire malaria transmission season. The study was carried out at the end of the *P. vivax* transmission season and at the start of the *P. falciparum* season; this might explain why

there was a larger treatment effect seen on *P. falciparum* transmission compared to *P. vivax* transmission. It is possible that if the study had been carried out longer, then a larger effect would have been observed. As *P. vivax* malaria is known to recrudesce, the study investigators should have cleared all malaria cases through an appropriate treatment regimen after checking for G6PD-deficient individuals[57] so that any cases that were observed would be classified as new malaria cases and not as recurrent *P. vivax* cases. The study used passive surveillance of malaria cases; consequently some cases not reporting to the health clinic might have been missed, lowering the sample size and power of the study to observe a treatment effect. This might explain why a treatment effect was not seen among females, because they were less likely to leave their homes due to the practice of purdah. In the evening, they might also have been using their *chaddars* as bedding for their children as a protective effect was seen only among those individuals <20 years of age. Compliance was established by visiting the households every 2 months. As frequent compliance inspection was not done compliance in between the months cannot be ascertained, and hence the findings of the study are less robust. As with all intervention studies, compliance is essential for an intervention to be considered effective, although the *chaddar* is a piece of clothing that is used on a daily basis.

A second single-blind RCT by the same group that investigated the effect of ITNs, insecticide-treated *chaddars* used to sleep in, and residual pyrethroid spraying of individual houses for the prevention of cutaneous leishmaniasis (CL) in Kabul, Afghanistan, also demonstrated a significant protective effect.[132] The incidence of CL among those randomized to the control was 7.2%, among ITN users 2.4% (OR: 0.31, 95% CI: 0.2–0.5), among impregnated *chaddar* users 2.5% (OR: 0.33, 95% CI: 0.2–0.6), and among those living in λ-cyhalothrin-sprayed houses 4.4% (OR: 0.60, 95% CI: 0.3–0.95). ITNs and impregnated *chaddars* were equally effective, providing about 65% protective efficacy, with approximately 40% protective efficacy being attributable to individual house spraying. The study was well powered: it was conducted in 1997–1998 among a nonimmune population of 3666 people over 15 months. New cases of CL were diagnosed based on clinical criteria diagnosed by the inspection of lesions, but parasitological confirmation could not be completed after aid organizations were ejected from Kabul in July 1988. Another difficulty of working in such a challenging environment was that compliance could not be measured, because spot checking would have invaded the privacy customs strongly upheld in the region. No significant differences for age or sex were found between new cases in the intervention and control groups. No serious side effects were reported, and interventions were generally popular; ITNs were the most popular, followed by residual spraying and then impregnated *chaddars*. Both ITNs and *chaddars* are useful in this region, as the population tends to be quite mobile. This population mobility caused massive loss to follow up (45%) as people moved out of the study area, but the study investigators had anticipated this and accounted for it during the recruitment of study participants. This demonstrates the importance of recruiting the appropriate sample size in any study.

A double-blind placebo-controlled trial to determine the efficacy of permethrin-impregnated uniforms among Iranian soldiers in Isfahan demonstrated a reduction in the odds of contracting CL. However, this effect was not significant, possibly because the study had only 134 people per treatment arm for 3 months of exposure in the field (1608 person-weeks per arm). Compliance was high, as the soldiers were required to wear the uniforms day and night and compliance was monitored. As compliance was ascertained, the results of this study may be credible. However, the method used for randomization was not described. This may have been done incorrectly, biasing the study and hence the observation of no treatment effect in the treatment arm. Both the participants and the study investigators were blinded to the treatments, reducing chances of selection bias.[133] The study, however, showed that permethrin-impregnated uniforms are safe for human use and no adverse effects were observed. Therefore, they present a potential tool that can be explored for malaria control. The fact that all the lesions (sites of infection) among the treated

uniform group were on sites unprotected by the uniform (face and wrist) is of importance; but in the control group, lesions were found on the arm and trunk. If the soldiers had been using full personal protection including a topical repellent for use on their face and hands,[13] they may not have contracted leishmaniasis.

In the Thailand–Cambodia border, a randomized placebo-controlled trial evaluating the effect of 2 gm permethrin per treated uniforms versus kerosene-treated uniforms on preventing malaria among the Royal Thai Army demonstrated no effect. The population was 403 male soldiers on active duty for 6 months. The randomization method was not outlined in this study, and compliance could not be established at all times. Both these factors could have confounded the findings of this study as the selected study participants might have had confounding characteristics. Also, as compliance could not be established both groups might not have used the repellent, therefore biasing the study toward the null hypothesis. One study arm may also have not complied with the intervention and similarly driving the effect toward the null.[59]

South America

A double-blinded placebo-controlled study in Colombia among 86 soldiers randomized to 600–712 mg/m^2 permethrin-treated uniforms and 86 soldiers randomized to water-treated uniforms over 4.2 weeks showed the uniforms to be 79% protective against malaria, 3% versus 14% among treated and control groups, respectively, and 75% protective against CL, 3% versus 12% among treated and control groups, respectively.[46]

The same double-blind RCT carried out in Colombia to determine the efficacy of permethrin-impregnated uniforms against both malaria and CL demonstrated a reduction in the RR of malaria (RR = 0.29, p = .015) and CL (RR = 0.21, p = .002).[46] As adherence to instructions to wear the permethrin-treated clothing day and night could not be monitored, the findings of this study are debatable, as with all studies in which compliance could not be established. However, the monitoring of disease was actively done every day and it is unlikely that any cases of malaria or CL could have been missed. There were very few reports on the adverse effects of insecticide-treated clothing. This is similar to other studies where very few adverse effects were reported, reinforcing the proposition that insecticide-impregnated clothing is safe for human use. This intervention can be applied to normal clothing, thereby tackling the problem of adherence so often seen when using topical repellents.

Sub-Saharan Africa

In a randomized community trial among 198 Somali refugees of all ages and both genders with no known allergies or respiratory problems at the Dadaab refugee camp, participants were randomized to either 0.37% permethrin or water placebo used to treated clothing and bedding, retreated every 3 weeks for a period of 3 months. All clothing and bedding was treated, including *diras*, saris, *jalbaabs*, *ma'awis*, shirts, sheets, and blankets. Use of the permethrin-treated clothing and bedding significantly reduced the odds of contracting malaria by 70% (CI was not reported).[62] Methods for randomizing treatments were described as systematic random sampling of households within treatment and control blocks 1.5 km apart, and compliance was maintained by regular retreatment of all clothing and bedding. The participants and laboratory technicians were blinded to the treatments. These aspects of the design are positive. However, the study was small and the statistical reporting was not good as it was unclear, it was overreliant on models, and p values and ORs were reported without CIs. However, the study reported the percentage positive in the treatment and intervention groups and the number of people tested, so these data could be used for a meta-analysis.

In another randomized community trial in Kenya to determine the effect of appropriate permethrin-impregnated clothing against malaria, it was found that the IRR of contracting malaria in those aged over 5 years in the intervention group was 0.187 (95% CI = 0.046–0.770, $p = .02$) compared to the control group.[134] For those under 5 years of age, however, no effect was seen. A total of 472 individuals were enrolled in a randomized community trial where the unit of randomization was the hamlet (*manyatta*) with 234 and 238 in the experimental and control arms, respectively. Baseline data included sociodemographic data, parasite prevalence data from thick and thin blood smears, and clinical measures of malaria. The intervention involved the dipping of *shukas* owned by the experimental group in permethrin, although the dose was not available in the publication. The prevalence of malaria in the study population (based on slide confirmation) was considerably lower than that used for the power calculation based on clinical estimates (2.2% vs. 20%). For those aged 6 or over, the rate of malaria cases (events per 10,000 person-days at risk) was 1.41 in the experimental group versus 7.49 in the control group (IRR: 0.187, 95% CI: 0.046–0.770). For children less than 5 years of age, results were imprecise with no clear benefit of the intervention. An attempt was made to impregnate all *shukas* of the experimental group. However, some children refused to have their *shukas* dipped in the cold early morning hours, as it was their only clothing. Other children, one-third of the 5 years and under in both groups, owned no *shuka*. The researchers had been aware of this before the study, but had felt that this would not affect results because preliminary research had indicated that the children without *shukas* slept under their mothers' *shukas* at night. Of the four cases that occurred in the intervention group, three did not own *shukas* and the fourth owned a *shuka* that was not impregnated. This incomplete coverage, coupled with the fact that the study investigators did not establish the local baseline incidence rate, led to an underestimation of the sample size required to observe a treatment effect. This shortcoming underlines the importance of establishing baseline factors before any study is implemented. Clinical reports implied that 35% of all patients were seen for malaria, and the clinicians' predicted prevalence of parasitemia was even higher (50%). Although a more conservative 20% was used to calculate sample size, the 2.2% parasitemia observed at baseline clearly reduced the statistical power of the study. This highlights the unreliability of malaria reports based on clinical diagnoses, which was also one of the reasons for the Tanzanian study of deet repellent being underpowered.

Other Studies

In a clinical trial in Myanmar, the use of treated scarves and hand bands were significantly associated with a lower incidence of malaria compared to the control arm where these interventions were not used.[135] The major local vector was *Anopheles minimus*, an outdoor and early evening biting mosquito. This makes treated scarves and hand bands appropriate control tools in this setting, as conventional tools cannot be used at these places and times. The study was carried out for a short period of time and did not take into account the low transmission season and was therefore not possible to establish the seasonal effect of this intervention. Compliance assessment was carried out in 10% of the study participants. From this sample, the compliance of the entire study population could be inferred. Also, the investigators carried out regular bimonthly checks on compliance and random spot checks. The compliance monitoring of this study was well conducted, and the results can be considered credible. The results from toxicity evaluations of this intervention did not demonstrate any adverse effect. This was in agreement with other studies that assessed the toxicity of insecticide-treated clothing.

All the earlier mentioned studies are associated with a protective trend of repellents against malaria. Most studies had questionable study designs and, therefore, the results of these studies could not be conclusively relied on. However, the fact that a protective trend was observed

in all of them reinforces the need to conduct a well-designed, large-scale trial to ascertain the effect of repellents on disease incidence.

MOSQUITO COILS

Randomized Controlled Trials in Southeast Asia

There have been two randomized trials evaluating the impact of burning mosquito coils every evening on malaria transmission, both conducted in Southeast Asia. The first study[81] was a single-blind, cluster-randomized comparative control clinical trial conducted in Ruili district, Yunnan province, People's Republic of China, close to the Myanmar border between April and October 2007. Yunnan is one of only two provinces in China that still has malaria cases and the Ruili district has a particularly high number of cases. The area is heavily forested, a high proportion of migrant populations moves over the border between countries, and it has many remotely located minority group habitations, making implementation of vector control and public health programs extremely difficult. All the communities enrolled were in rural areas.

The trial was designed to measure and compare the protection against malaria provided by mosquito coils, LLINs, or a combination of the two. The study recruited 2052 households comprising 7341 individuals, excluding individuals under 6 years and pregnant women. Households were randomized into one of four groups: coils (0.03% transfluthrin coils, SC Johnson), deltamethrin LLINs (TianJin-Yorkool, Ltd., Tianjian, People's Republic of China; and Lantrade Global Supplies, Ltd., Gerrards Cross, United Kingdom), coils plus LLINs, and a control group without any intervention other than whatever control intervention they were already using. At baseline and every month post intervention, each individual was actively screened for malaria (both *P. falciparum* and *P. vivax*) by RDT. At the end of the 6-month study, there were 69 confirmed malaria cases in the control group, 16 in the coil group, 14 in the LLIN group, and 5 in the combined coil plus LLIN group. In the coils-only group, the age-adjusted OR for *P. falciparum* malaria was 0.23 (95% CI = 0.11–0.50, $p = .0002$) and protective efficacy against *P. vivax* was 80%, OR = 0.20 (95% CI = 0.09–0.44, $p < .0001$), and were not significantly different from those for LLINs or LLINs plus coils. The level of compliance with the allocated interventions was high: it was noted that >94% of individuals used coils and/or LLINs for >90% of the month prior to the surveys. Conversely, those in the control arm were less compliant, with 13%–19% using local coils for 3 or more days per month. A per-protocol analysis including only those with >90% compliance gave almost identical results to the intention-to-treat analysis.

A second, more recent double-blind, placebo-controlled cluster-randomized trial conducted in Sumba, Indonesia, to evaluate the effect of 0.0097% metofluthrin mosquito coils only (no LLINs were used in either study arm) against malaria[79] comprised two clusters (1000 people) allocated to the treatment arm and two clusters (1000 people) allocated to the control arm. Of these, 45 healthy males who were >17 years, >40 kg, G6PD normal, and resident in the village for the study period in two clusters per arm ($n = 90$ per arm) were followed up as the study cohort for 26 weeks. Compliance with mosquito coils was monitored daily and malaria was monitored weekly among participants by active case detection. In addition, malaria vector abundance and biting time was measured by indoor and outdoor human landing catch; vector population age was estimated from parity rates by detinova ovarian dissections, and sporozoite rate in vectors was measured by CSP-ELISA (circumsporozoite protein enzyme-linked immunosorbent assay). Malaria incidence among the treatment group was 0.904 versus 2.324, which equates to a 61.1% protective efficacy (95% CI = 37%–75%, $p < .00001$).

CONCLUSIONS

These two trials[79, 81] of spatially acting pyrethroids used as mosquito coils were tested in isolation, without the addition of LLINs, and provided unambiguous evidence that individual malaria risk is significantly reduced by >60% simply through avoiding mosquito bites. These trials were conducted under rigorous conditions that should set the benchmark for future trials, because they were designed, powered, and analyzed with the help of a statistician; had adequate randomization; were placebo controlled, allowing adequate blinding[11]; and used active case detection with RDTs with polymerase chain reaction confirmation throughout the study. In addition, essential to the success of any repellent study, very high compliance was observed throughout, which was carefully monitored by study staff. Furthermore, both studies were conducted in suitable field sites. In both cases, a large proportion of mosquito bites occurred before bedtime (Table 7.1) and mosquito coils were culturally acceptable (a smoky environment is tolerated). Furthermore, repellents may be more effective in Southeast Asia because malaria transmission is low and seasonal and the main malaria vectors are opportunistic and will feed on other hosts.

Future trials should attempt to match the high standards of these trials and also include some further information on community-level measurements of the impact of mosquito coils on malaria vector population dynamics. These data were collected in some extremely detailed studies on dichlorvos during the 1960s and showed that at a high enough coverage of repellent interventions there can be a community protection demonstrated by decreased human–vector contact, vector infectiousness, and vector longevity.

This is the key piece of information that should be collected from any future trials of personal protection tools if they are ever to be considered as public health tools applied at a community scale to prevent disease transmitted outdoors, in the day or evening, rather than just niche tools for particular lifestyles or occupations. Furthermore, dichlorvos is an example of a repellent tool that requires little compliance—it just requires the replacement of dispensers every 2 weeks. It is essential that future research examines such low-compliance interventions that will help to address the two greatest barriers to repellent implementation: cost and compliance.

Findings from the review strongly support the theory that use of repellents has a beneficial protective effect against transmission of disease, mainly, malaria and leishmania as very little data are available on dengue. Even though individual studies had varying outcomes, the combination of all the available evidence does support the notion that specific repellents should be incorporated into current vector control strategies where appropriate. We recommend the use of repellents (both spatial and topical) at times when current control measures cannot be implemented. The other key finding from this review was the paucity of existing high-quality data. To improve the speed at which products are developed and approved by bodies such as the WHO, there is a clear need for harmonization of methodologies and outcomes measured in new trials and evaluation of vector control tools, in particular, the way they are reported. Researchers need to be encouraged to ensure that their piece of research contributes to the overall picture in a research field. Clear reporting of outcomes and use of guidance available for this task, for example, using CONSORT guidelines,[136] should make future trials more robust and data easier to assimilate by means such as systematic review and meta-analysis for use by policy makers. It was also clear from this review that those trials collecting data through active case detection were far more powerful than those using passive case detection. Important secondary end points of any trial are entomological correlates of reduced infection, that is, human–vector contact, parity rate, sporozoite rate through regular human landing catches, and human compliance with the intervention. An exposure-free measurement of human landing is especially needed for large-scale epidemiological work particularly in areas where dengue or other arboviruses are prevalent. Measurements of compliance such as salivary antigen markers of exposure to mosquito bites[137] are a key research need for rigorous and ethical research into disease prevention using vector control tools as the markers of exposure may be used as a measure of both exposure and compliance.

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PART II

Development

CHAPTER 8

Testing Methods for Insect Repellents

Louis C. Rutledge, Zia A. Mehr, and Mustapha Debboun

CONTENTS

HISTORICAL TRENDS IN TEST METHODS

The use of insect repellents began in prehistoric times among our prehuman and prehistoric human ancestors. Many animals, including primates, are known to apply substances derived from plant and animal sources to the integument for protection against predators and ectoparasites.[1] The ethnobotanical traditions of preliterate human societies include the use of plant, animal, and inorganic materials to repel insects.[2] It seems probable that the test method used in prehistoric times was that of simply observing the repellent effects of fortuitous contact with a plant, animal, or inorganic substance, followed, perhaps, by simple trial-and-error experiments. The knowledge acquired was transmitted to succeeding generations by demonstration and imitation and in human societies through oral communication. Over time, the accumulated knowledge of repellents became incorporated into local folklore and sometimes into language itself, as in the words "fleabane" and "lousewort."

Early History of Insect Repellents

Repellents entered historical records with the invention of writing, which first occurred in ancient Sumer about 5000 years ago.[3] Inasmuch as documentation of extant knowledge is integral to the scientific method, the invention of writing can be considered a further important advance in testing methodology. The first recorded use of insect repellents was the reference by Homer (c. 900–800 BCE) to "pest-averting sulfur."[4] In China, the *Chou Kuan*, written during the Chou dynasty (1030–221 BCE), described methods for treating seeds with insecticides and insect repellents.[5] The Greek pharmacologist Dioscorides (c. 40–90 CE) included information on repellents in his *De Materia Medica*, which remained the leading pharmacological text for 16 centuries.[6]

Many other ancient writers recorded contemporary knowledge and beliefs about insect repellents in their writings. The bulk of these ancient writings on repellents are brief comments in larger works on topics such as agriculture (Virgil, 70–19 BCE), medicine (Hippocrates, 460–377 BCE; and Avicenna, 980–1037 CE), and botany (Theophrastus, 372–287 BCE). The Roman Pliny the Elder (23–79 CE) recorded numerous insect repellents, both valid and apocryphal, in his encyclopedic *Naturalis Historiae*, which became the standard text for general education in the European Middle Ages.[7] In Book I of *Naturalis Historiae*, Pliny listed the names of numerous writers from whose writings he had obtained the information contained in each of the remaining 36 books.

The invention of modern printing by Johannes Gutenberg (1390–1468) was a further advancement, which permitted inexpensive dissemination of ancient writings and introduced the era of printed herbals.[8,9,10] These herbals provided for the first time accurate, detailed descriptions and illustrations of medicinal plants, including some that produce insect-repelling substances (Figure 8.1). However, some herbalists used the fallacious doctrine of signatures (notably, William Cole, c. 1626–1662) and/or the teachings of astrology (Paracelsus, 1493–1541) to infer the practical uses of the plants shown and described. According to the doctrine of signatures, the phenological and morphological characteristics of a plant indicate its practical uses. For example, the heliotrope was thought to be a treatment for scorpion stings because its curved inflorescence resembles the metasoma of a scorpion, which bears the sting. Some classical herbals have been reprinted, in whole or in part, in modern times (Figure 8.2),[11,12] and many popular modern herbals have appeared since the original herbals were produced.[13]

Early Modern Period of Insect-Repellent Testing

The rise of modern science and the Industrial Revolution signified the beginnings of a true science of insect repellents. New sources of insect repellents and insecticides were found in exotic plants and in derivatives of plant resins, coal, and petroleum. Some of the resulting repellent products were documented and illustrated by Gittins and Trask[14] in 2005 (Figure 8.3). The popular literature of the time included a genre of books, sometimes called "receipt books," that collected directions for compounding various domestic and industrial materials, including repellents.[15,16] Many repellents were also described in patent documents, which were available to the public from the government. For example, berries of the wingleaf soapberry (*Sapindus marginatus*) and preparations of the berries were patented as repellents for insects in stored food.[17] The cinchona alkaloids, as a class, were patented as repellents for the clothes moth.[18] The genus *Cinchona* (Rubiaceae) includes about 38 species known as sources of quinine and related compounds. In addition, a class of pharmacological books, variously called *Materia Medica*, formularies, dispensatories, or pharmacopoeias, commonly included insect repellents as medicines.[19,20] With the exception of patent documents, these publications typically did not provide any test data or any information on the test methods used to support the claims and assertions made for the repellents described.

In the years following 1900, the study of insect repellents became a mature science, on which scientific journals published not only the materials tested but also the methods used and the data obtained. The state of the science for repellents intended for use on humans was summarized in 1912 by Howard et al.[21] Several of the repellents mentioned by them, including citronella oil, cedar oil, and pyrethrum, are still in use. The state of the science for repellents intended for use on livestock was summarized in 1914 by Graybill.[22] Again, several of the materials mentioned in this work, including citronella oil, pyrethrum, and tobacco powder (nicotine), are still in use. The 1919 study by Bacot and Talbot[23] can be taken as a representative of the research done on repellents in this early period. The study was conducted in the laboratory using the yellow-fever mosquito *Aedes aegypti* (Linnaeus) as the test insect and the authors as test subjects. A measured dose of the test

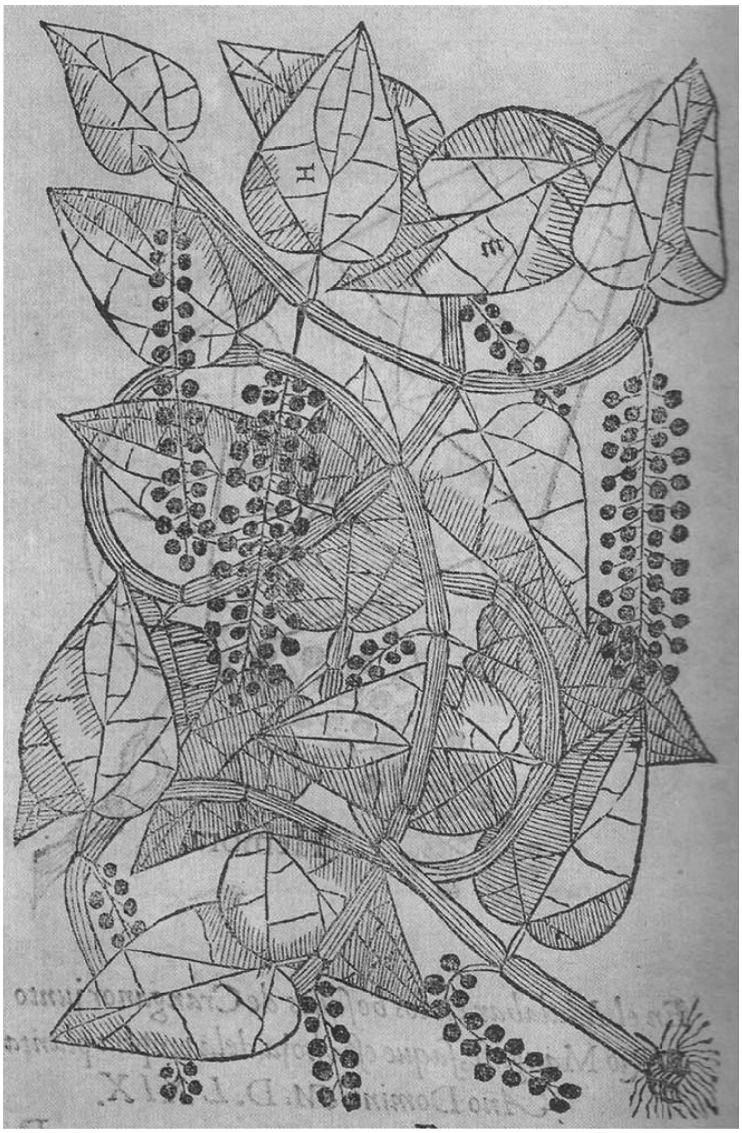

Figure 8.1 Pepper (*Piper nigrum*), the source of piperine, as shown in a 1578 herbal by Christoval Acosta. (From Arber, A., *Herbals: Their Origin and Evolution*, Cambridge University Press, Cambridge, United Kingdom, 2010.)

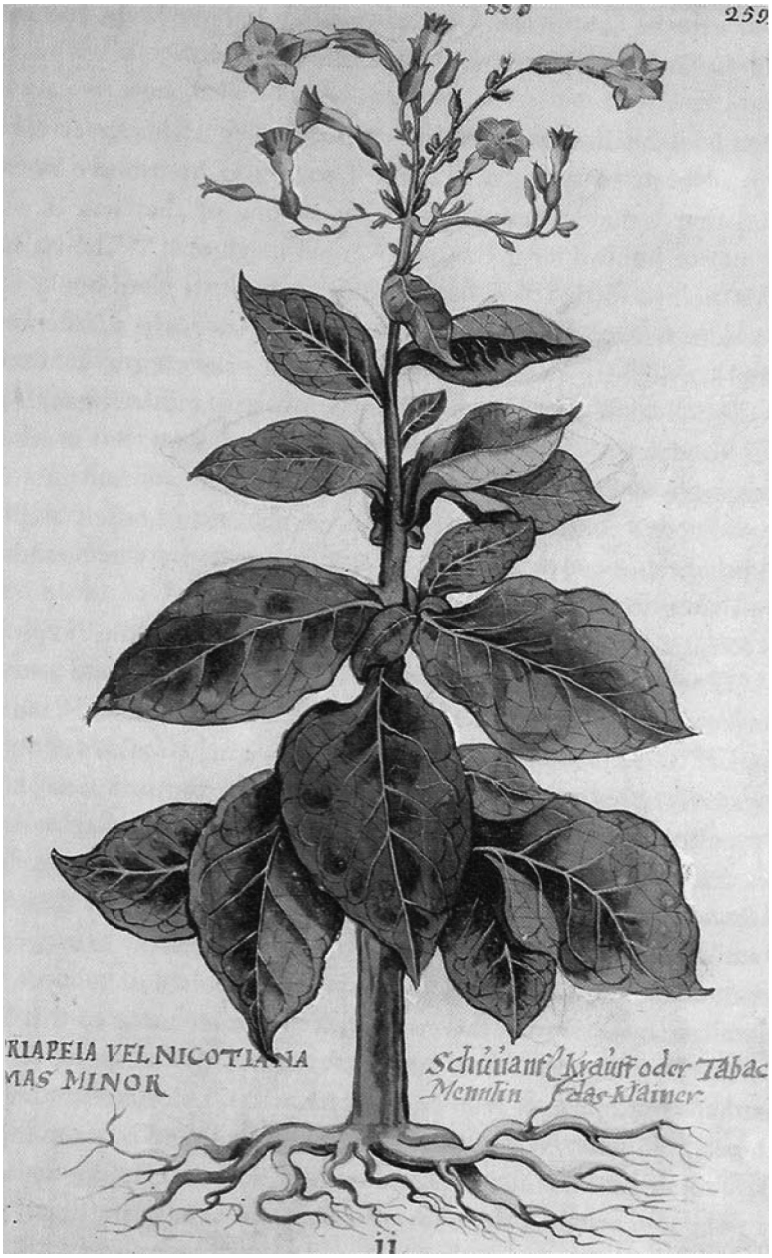

Figure 8.2 Tobacco (*Nicotiana tabacum*), the source of nicotine. Nicotine was formerly used as a repellent, as well as an insecticide. (From Pavord, A., *The Naming of Names: The Search for Order in the World of Plants*, Bloomsbury Publications, New York, 2005.)

material was applied to one forearm of a test subject, and the alternate forearm was left untreated as a control. After a predetermined test period, the treated forearm and the untreated forearm were exposed in turn to the test insects and the number of bites received on each was recorded. The data obtained were interpreted as the percentage of the number of bites received on the treatment with respect to the number of bites received on both the treatment and the control.

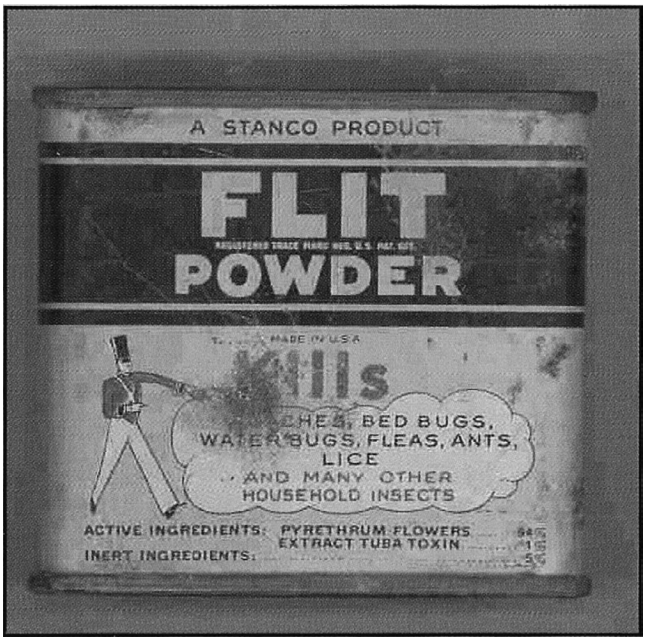

Figure 8.3 Antique container of pyrethrum powder. Labeled here as an insecticide, pyrethrum powder was also used as a repellent. (From Gittins, J., and Trask, B.H., *Wing Beats*, 16 (4), 16–20, 2005.)

Recent Trends in Testing Insect Repellents

It should not be inferred from any of the foregoing that the science of insect repellents was a backward science, as both the science of animal behavior in general and that of insect behavior in particular were at that time in a formative state.[24,25] As will be seen in the remaining sections, those early pioneering methods were extensively refined, elaborated, extended, and modified in the succeeding years of the twentieth and early twenty-first centuries.

Test Materials

Historically, the number of materials available to investigators for testing as insect repellents has grown with the growth of organic chemistry. The essential oils of many known and newly discovered plants and many derivatives of plant resins (turpentine), coal (creosote), and petroleum (kerosene) were shown to have insect-repelling properties. Fractionation of these materials and purification of the constituent compounds further increased the number and variety of potential insect repellents available for testing. The discovery of these compounds, in turn, led to the synthesis of entirely new compounds not known to occur in nature. The availability of large numbers of compounds for testing prompted the development of screening tests designed to quickly and efficiently identify the compounds having significant repellent properties. In the latter half of the last century, the U.S. Department of Agriculture (USDA) published three voluminous compendiums of the results of screening tests conducted within the USDA, representing the most notable achievement in the screening of potential insect repellents to date.[26,27,53] Table 8.1 shows some of the notable repellents screened by the USDA from 1942 to 1977.

Current insect-repellent development programs are in general more selective in the choice of test materials than the random screening programs of the past. There is an ongoing revival of interest in repellents of botanical origin, reflecting the public mistrust of synthetic chemicals. There has been

Table 8.1 Notable Repellents Screened by the USDA, 1942–1977

Repellent	Primary Uses	References
Allethrin	Mosquito coils	53
Benzyl benzoate	Clothing treatment	26, 53
Butopyronoxyl	Topical repellent	53
2-Butyl-2-ethyl-1,3-propanediol	Clothing treatment	53
Cedar oil and derivatives	Clothes moth repellent	53
Citronella oil and derivatives	Topical repellent, repellent candles	53
Deet	Topical repellent, clothing treatment	53
Dibutyl phthalate	Clothing treatment	53
Dimethyl carbonate	Topical repellent	53
Dimethyl phthalate	Topical repellent, clothing treatment	53
Ethyl hexanediol	Topical repellent	53
Eucalyptus oil and derivatives	Topical repellent	26, 53
MGK Repellent 326	Topical repellent	53
MGK Repellent 874	Cockroach repellent	26
Naphthalene	Clothes moth repellent	53
p-Dichlorobenzene	Clothes moth repellent	53
Pyrethrum and derivatives	Mosquito coils, topical repellent	53

recent research on and development of finishing processes to incorporate insect-repellent materials into textiles at the point of manufacture and of controlled-release formulations of topical repellents that extend the period of effectiveness of the active ingredient, limit its absorption through the skin, and improve its cosmetic properties. There have been recent studies on the prospects for designer repellents, that is, new chemical compounds specifically designed and synthesized to fulfill the efficacy, persistence, safety, and cosmetic requirements for improved insect repellents. It is not yet clear how these ongoing developments may lead to modifications in the current methods of testing repellents.

Test Arthropods

Historically, the yellow-fever mosquito has been the preferred test insect for laboratory tests of insect repellents, because of its medical importance; its adaptability to laboratory conditions; and the extensive data available in the literature on its biology, physiology, genetics, and behavior. However, as species and strains of arthropods differ significantly in their tolerances to repellents,[28] additional test species are necessary to obtain a general result regarding any particular repellent. In practice, laboratory test species are usually selected on the basis of medical or veterinary importance and the availability of established laboratory colonies. Besides the yellow-fever mosquito, the most notable laboratory test species are the lone star tick *Amblyomma americanum* (Linnaeus), mosquito *Anopheles stephensi* (Liston), sand fly *Phlebotomus papatasi* (Scopoli), and oriental rat flea *Xenopsylla cheopis* (Rothschild).

Field tests of repellents may target a major pest or vector species, such as *Anopheles gambiae* (Giles), *Aedes aegypti* L., or *Culex quinquefasciatus* (Say), as part of ongoing research on that species, but programs dedicated specifically to the research and development of insect repellents also select field test species on the basis of local availability, as well as medical and/or veterinary importance. Historically, *Anopheles quadrimaculatus* (Say), *Aedes taeniorhynchus* (Wiedeman), and *Aedes vexans* (Meigen) have been frequently used as field test species in the eastern United States.

In Vitro Test Methods

Numerous methods for testing repellents and attractants against insects in vitro were described in the course of the last century. Table 8.2 provides an outline of the many materials and methods

Table 8.2 Representative Materials and Methods Used in the In Vitro Studies

Test Materials
Physical state: gas, liquid, solid
Composition: inorganic, organic
Sources: natural (powder, extract), synthetic (derivative, de novo)
Formulations: simples, solutions, mixtures
Test Insects
Classification: Thysanura, Isoptera, Blattodea, Hemiptera, Phthiraptera, Coleoptera, Hymenoptera, Lepidoptera, Siphonaptera, Diptera, Acari
Significance: medical/veterinary, household/structural, stored products, agricultural/horticultural, experimental (e.g., *Drosophila melanogaster*)
Test Population
Setting: laboratory, field
Size: one to thousands
Experimental Design and Data Analysis
Qualitative observations
Quantitative observations: replication, raw data, descriptive statistics, indexes
Controls, material standards, paired observations
Bioassays
Apparatus
Size: centimeters to meters
Shape: cylindrical/tubular, conical, rectilinear, complex
Materials: wood, cardboard, plastic, metal, glass, fabric
Attractor: light, bait, host plant/animal
Temperature, humidity, and illumination: ambient, controlled, integral
Airflow: static air (diffusion), ambient air (convection), forced airflow (ambient air, conditioned air)
Recording Media
Manual: notebook, data sheet, check sheet, squared paper
Planimetric, photographic, radiometric, electrophysiologic
Recording Methods
Continuous recording
Time sampling: instantaneous sampling, one-zero sampling
Measures of Effectiveness
Locomotory behavior: chemotaxis, chemokinesis
Population size: trapping, dragging, oviposition data
Feeding behavior: landing, probing, biting, feeding
Products: excreta, frass
Effects: damage/weight loss (product, structure, host plant)

Sources: Rutledge et al., *J. Med. Entomol*., 14, 536–541, 1978; Dethier, V.G., *Chemical Insect Attractants and Repellents*, The Blakiston, Philadelphia, PA, 1947; Shepard, H.H. (ed.), *Methods of Testing Chemicals on Insects*, Vols. 1 and 2, Burgess Publishing, Minneapolis, MN, 1958–1960; Peterson, A., *Entomological Techniques: How to Work with Insects*, Edwards Bros., Ann Arbor, MI, 1964; Busvine, J.R., *A Critical Review of the Techniques for Testing Insecticides*, Commonwealth Agricultural Bureaux, Farnham Royal, England, 1971; Kennedy, J.S., Behaviorally discriminating assays of attractants and repellents, in *Chemical Control of Insect Behavior: Theory and Application,* H.H. Shorey and J.J. McKelvey (eds.), Chapter 13, Wiley, New York.

used. Three main streams in the development of in vitro test methods are discussed in the following subsections: olfactometers, alternative choice test systems, and in vitro blood-feeding test systems. Numerous methods are not easily categorized under any of these headings. Most of these methods were ad hoc methods, designed for use in individual studies or programs of research. Collectively, they provide an extensive history of principles, approaches, techniques, and findings in the study of insect attractants and repellents. For an introduction to the extensive primary literature, see the compilations by Dethier,[29] Shepard,[30] Peterson,[31] Busvine,[32] and Kennedy.[33]

Olfactometers

Perhaps the earliest olfactometer designed for the study of insect behavior is the Y-tube olfactometer designed by Barrows in 1907.[34] This basic design of olfactometer is still in frequent use, primarily in studies of insects of agricultural importance. Numerous additional types of olfactometers have been devised since then. Dethier[29] distinguished two basic types: the venturi type and the Y-tube type. In venturi-type olfactometers the composition and concentration of the test material in the olfactometer airstream are known, but in Y-tube-type olfactometers only the test material's composition is known.

Olfactometers designed for testing mosquito repellents have evolved into large, sophisticated test systems, incorporating subsystems for air intake, flow, conditioning, purification, and exhaust, and for photographic or electronic recording of test data. The olfactometers by Schreck et al.,[35] Kellogg et al.,[36] and Sharpington et al.[37] are representative of the more advanced designs. The design of Schreck et al. features two separate rectilinear test cages leading to separate traps to allow paired observations. Airflow is provided by a forced-air system with controls for flow rate, temperature, and humidity of the two separate airstreams. Provision is made for sampling composition, temperature, humidity, and flow rate of the airstreams when the olfactometer is in use. The airstreams pass over two separate liquid/solid test materials or, alternatively, over the treated/untreated forearms of a test subject before entering the test cage containing the test insects. The test insects fly upstream into the respective traps in response to the warm, humid airstream and/or emanations from the forearms of the test subject. After an allotted time, the cages are removed from the apparatus and the number of mosquitoes trapped is determined visually (one-zero time sampling).

The designs of Kellogg et al. and Sharpington et al. differ from that of Schreck et al. not only in detail but also in other respects. Both were designed specifically for the in vitro biological assay of repellents, and neither is designed to accommodate the forearms of a test subject. The design of Kellogg et al. features a single cubical test cage through which two separately conditioned airstreams are passed, a "background" stream (25°C and 45% relative humidity [RH]) and a "host" stream (35°C and 65% RH). A notable feature of this olfactometer is that the test material is injected directly into the host airstream by a screw-driven hypodermic syringe geared to provide a virtually unlimited range of injection rates. The concentration of the test material in the host airstream can be calculated precisely from the known flow of the host airstream and the known rate of injection of the test material, which is the advantage claimed for venturi-type olfactometers by Dethier.[29] The test insects fly up the host stream in response to the warmer, more humid air to land on the screened wall of the test cage within the confines of the host stream. Five counts of the number of test insects within this area are made at 15-second intervals (instantaneous time sampling).

The design of Sharpington et al. features a rectilinear wind tunnel longitudinally divided into four separate bioassay chambers. Each bioassay chamber is fitted on its upstream end with a 4 cm diameter glass cylinder that is closed with chick skin on its interior (bioassay chamber) end and connected to a constant-temperature water circulation system set to 34°C on its exterior end. Airflow is provided by a forced-air system that controls flow rate, temperature, and humidity. Notably, the airstream is purified by passing it through activated charcoal and zeolite filters, in addition. Glass partitions between the bioassay chambers separate the conditioned air into four separate odor

streams and prevent them from mixing as they pass over the four treated/untreated chick skins and through the four bioassay chambers to an exhaust outside the room. The test insects fly upstream in the respective bioassay chambers in response to the heat, humidity, and odor emanating from the respective chick skins. Individual video recorders continuously image the test insects on each skin for 10 minutes. Ten counts at 10-second intervals are subsequently made from the recording of the number of test insects present on each chick skin (instantaneous time sampling). The olfactometer was designed and operated for testing a control and three dose levels of the test material in bioassays. However, it is obviously equally adapted for use in experimental designs such as the 2 × 2 Latin square and factorial and binomial designs as well.

Alternative Choice Test Systems

An alternative choice test system is a treated/untreated enclosure or an assembly of such enclosures such that the test insect may freely enter into, remain in, or exit from a repellent-treated or -untreated enclosure during the test. Some designs do not permit reentry into an enclosure previously occupied. The name "alternative choice test system" does not imply that other test systems do not require alternative choices on the part of the test insect.

Perhaps the earliest test system of this type was that designed by Wigglesworth[38] in 1941 for use in studies of the human body louse. In succeeding years, numerous additional designs were described, primarily for use in the study of attractants and repellents for cockroaches and insects of agricultural importance.[29] The alternative choice test system design of J. S. Kennedy[39] published in 1947 (Figure 8.4) initiated a period of development of alternative choice test systems designed specifically for use in the study of the behavioral responses of mosquitoes to insecticides. From a scientific point of view, the distinct advantage of alternative choice test systems is that they permit the integrated study of the repellent, irritant, and toxic effects of the test material simultaneously. The most recent alternative choice test systems developed to date are those of Chareonviriyaphap et al.[40] and Grieco et al.[41] (Figure 8.5).

The test system of Chareonviriyaphap et al.[40] features a test cage that can be disassembled for cleaning, transportation, and storage. The four rectangular sides of the test cage are made of metal screening in acrylic plastic frames. One end of the test cage is closed with a removable acrylic plastic square fitted with a rubber valve to allow insertion and removal of test insects with an entomological aspirator. The opposite end is closed with a removable acrylic plastic square having a cutout providing access to an attached, wedge-shaped, stainless steel exit passage. Before use, the four sides of the test cage are connected, four treated or untreated test papers are clipped to the four sides, the two end pieces are inserted, the exit passage is blocked with a Styrofoam plug, and the cage is loaded with 25 mosquitoes to be tested. The test cage, so prepared, is then placed inside a closely fitting stainless steel cover consisting of four rectangular sides and two square end pieces, one of which has a rectangular cutout to accommodate the exit passage. The purpose of the stainless

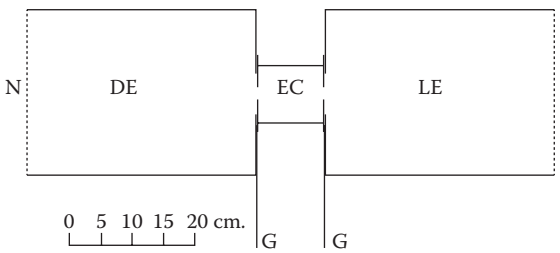

Figure 8.4 Alternative choice test system designed by J. S. Kennedy in 1947 for studies of the excitant and repellent effects of DDT. N, mosquito netting; DE, LE, escape chambers; EC, exposure chamber; G, glass plates. (From Kennedy, J.S., *Bull. Entomol. Res.*, 37, 593–607, 1947.)

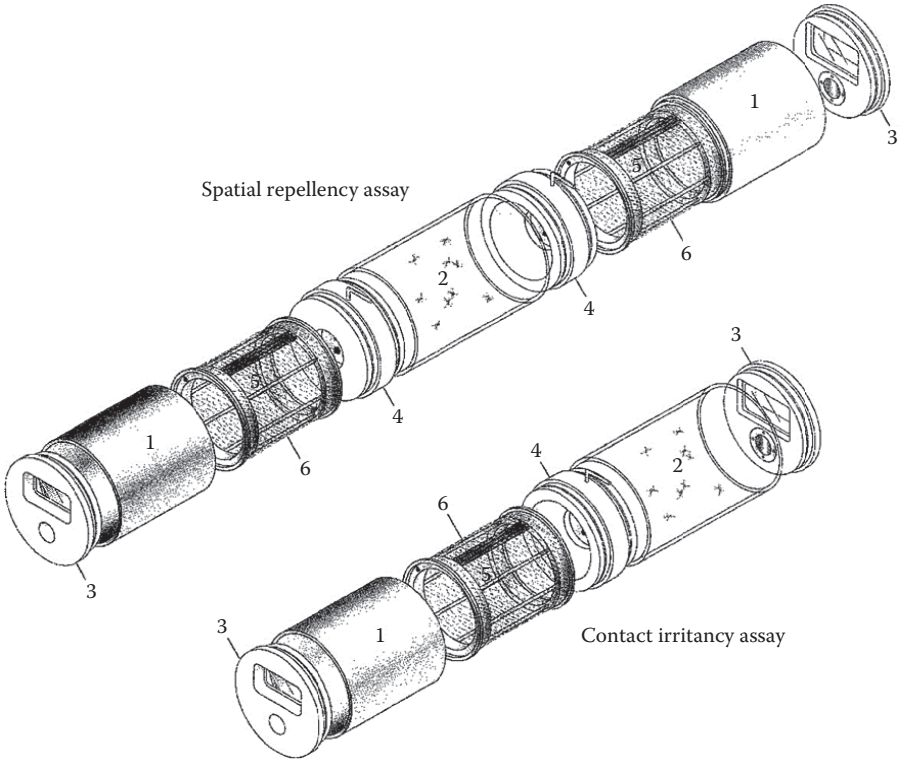

Figure 8.5 Advanced alternative choice test system. (From Grieco et al., *J. Am. Mosq. Control Assoc.*, 21, 404–411, 2005.)

steel cover is to exclude all light from the test cage except that entering through the exit passage. After a 3-minute waiting period, the plug is removed from the exit passage and a cardboard collecting cage is connected to the exit passage. The top of the collecting cage is screened to permit light to enter the exit passage and to allow visual counts of the numbers of mosquitoes that have escaped from the test cage. Counts are made at 1-minute intervals during a 30- or 60-minute test period (one-zero time sampling).

A notable feature of the Chareonviriyaphap test cage is that the test papers can be clipped to either the inside (the screened side) or the outside (the open side) of the four sides of the test cage. In the former configuration the test insects are exposed to physical contact with the test papers, whereas in the latter configuration the test insects are prevented from physical contact with the test papers by the intervening screens. The latter configuration allows the test cage to be used to study the olfactory effects of the test material separately from the combined olfactory and irritant effects. In the terminology and notation of factorial design, four treatments are possible: treated papers, contact allowed (a_1b_1); treated papers, contact not allowed (a_1b_2); untreated papers, contact allowed (a_2b_1); and untreated papers, contact not allowed (a_2b_2). Each combination of species, material, and dose of interest is tested in each of the four test cage configurations, and the time series of visual counts is analyzed as a biological assay to estimate the median effective time (ET_{50}) and/or similar estimates of the time taken by the test insects to escape the test cage.

The test system of Grieco et al.[41] is composed of two aluminum treatment cylinders with removable inserts, two polyacetal plastic linking sections, a clear acrylic plastic cylinder, and two

polyacetal plastic end caps (Figure 8.5). The insert of a treatment cylinder is a cylindrical aluminum framework around which a layer of treated/untreated nylon netting is wrapped, the whole of which fits closely within the treatment cylinder. The two linking sections allow the plastic cylinder to be connected end to end with one or both treatment cylinders. Each linking section is milled to provide a conical exit passage that can be oriented either toward or away from the adjacent treatment cylinder. The aperture of the exit passage is fitted with a butterfly valve that can be adjusted to allow or prevent the passage of test insects. The clear plastic cylinder and the two end caps are fitted with openings for insertion and removal of test insects, and the end caps are also fitted with windows for observation of the interior.

A treatment cylinder with its insert and two end caps are used in testing materials for toxicity. Twenty mosquitoes are exposed to treated/untreated netting inside the treatment cylinder for 1 hour, after which the resulting mortality and knockdown are determined. Surviving mosquitoes are then transferred to holding cages for determination of mortality and knockdown at 24 hours. The significance of differences among treatments is inferred by analysis of variance. An assembly of a treatment cylinder with its insert, a linking section with the aperture oriented away from the treatment cylinder, the clear plastic cylinder, and two end caps is used in testing materials for irritancy to the test insects. Ten mosquitoes are exposed to treated/untreated netting inside the treatment cylinder for 30 seconds, after which the butterfly valve is opened to allow the mosquitoes to escape into the clear plastic cylinder. After an additional 10 minutes, the butterfly valve is closed and the number of mosquitoes present and knocked down or present and not knocked down is recorded for each cylinder (one-zero time sampling). The significance of differences among treatments was inferred by signed rank tests (n.b., it seems that the test system in this configuration would measure the combined irritant and olfactory effects of the test material, as the probability of both physical contact with the test material and olfactory contact with the vapors of the test material would exist in the treatment cylinder.) An assembly of two treatment cylinders with inserts (one with treated netting and one with untreated netting), two linking sections with apertures oriented toward the adjacent treatment cylinders, the clear plastic cylinder, and two end caps is used to test materials for vapor repellency to the test insects. Twenty mosquitoes are placed in the clear plastic cylinder, and the cylinder is darkened with an opaque cloth. After 30 seconds, the butterfly valves are opened to allow the mosquitoes to enter the treatment chambers in response to light from the windows in the two end caps. After 10 minutes, the butterfly valves are closed and the number of mosquitoes present and knocked down or present and not knocked down is recorded for each cylinder (one-zero time sampling). The significance of differences among treatments was inferred by signed rank tests.

In Vitro Blood-Feeding Test Systems

In vitro blood-feeding test systems provide test insects with drawn blood covered with or contained within treated/untreated skin or a skin surrogate such as goldbeater's skin. Such test systems are thought to be more appropriate than other in vitro test systems for testing topical insect repellents because they directly engage the natural blood-feeding instincts of the test insects.

Bar-Zeev and Smith[42] described the first in vitro blood-feeding test system for repellents in 1959 (Figure 8.6). The Bar-Zeev and Smith test system was a small-cage, no-choice test system in which the test insects were fed through *baudruche* (goldbeater's skin) on blood warmed in an incubator. Subsequently, Rutledge et al.[43] described a large-cage, multiple-choice test system in which the test insects were fed through *baudruche* on blood warmed by a constant-temperature water circulator (Figure 8.7). The latter test system has been used effectively by several institutions in tests of repellents against several species of mosquitoes. A comparison of the results obtained using this test system with those of comparable tests on the human forearm was provided by Rutledge and Gupta[44] in 2004.

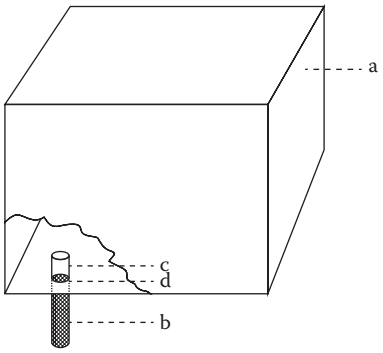

Figure 8.6 In vitro blood-feeding test system. (a) Incubator, (b) cage, (c) glass tube containing blood, and (d) membrane. (From Bar-Zeev, M., and Smith, C. N., *J. Econ. Entomol.*, 52, 263–267, 1959.)

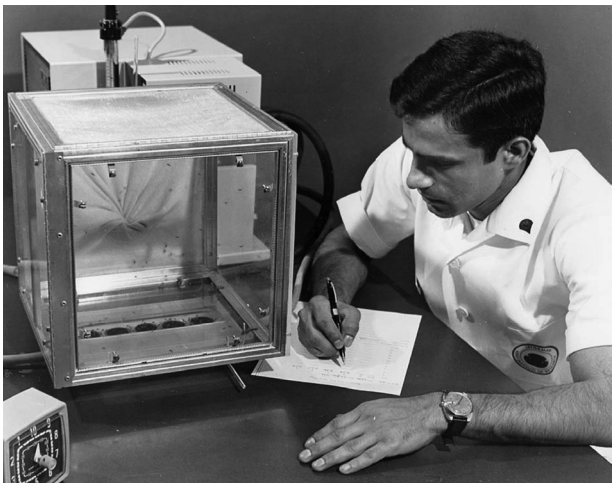

Figure 8.7 In vitro blood-feeding test system. (From Rutledge et al., *Mosq. News*, 36, 283–293, 1976.)

Animal Models

The first recorded tests of repellents intended for human use using animal test subjects were the tests conducted by Kawamura[45] against chigger mites using rabbits, guinea pigs, and monkeys in the field in Japan in 1926. Since then, most tests using animals have been conducted on laboratory animals against laboratory-reared or field-collected insects.[46] To date, tests of repellents have been conducted on experimental animals against various soft ticks, hard ticks, assassin bugs, pulicid fleas, sand flies, mosquitoes, stable flies, and tsetse flies. The most frequently used test insect has been the yellow-fever mosquito, and the most frequently used experimental animals have been the rabbit, guinea pig, and white mouse. Comparisons of results obtained using laboratory rabbits and mice with those of comparable tests on the human forearm have been provided by Rutledge et al.[47,48]

Biomedical research using animal subjects has both advantages and disadvantages in terms of scientific ethics and relevance. In vitro test systems are not normally regulated, but government approval for the use of animal test systems is required. Historically, cruelty was a common feature of animal experiments, and the laws and regulations followed today were adopted to implement

humanitarian values in biomedical research. The policies, principles, and procedures governing experimentation on animals in the United States are explained in the *Guide for the Care and Use of Laboratory Animals*[49] published by the National Academies Press, Washington DC. Although the procedures required impose a significant burden on research and development, the burden is substantially less than that imposed by the procedures required for equivalent research and development using human test subjects.

Because of interspecific differences between animals and humans, results obtained in tests on animals cannot be directly equated with results that would be obtained in comparable tests on humans. With respect to topical repellents, these interspecific differences relate to the temperature and permeability of skin, skin's blood content and flow rate, sweat and sebaceous glands, and density and length of hair. In principle, several approaches to the solution of these difficulties are possible: (1) selection of test species that differ least from humans with respect to relevant anatomical and physiological parameters, for example, body temperature, which is correlated with skin temperature; (2) use of juveniles of species in which the young are born hairless; (3) use of breeds or strains having desirable traits such as hairlessness; (4) shaving the test animal; (5) pharmacological control of relevant parameters such as sweating and body temperature; (6) regulation of the ambient conditions of the test, for example, regulation of ambient temperature to produce a relevant skin temperature in the test animal; and (7) statistical adjustment of the test data using correction terms, correction factors, and/or curve fitting procedures. Historically, most of the foregoing principles were demonstrated by researchers in the course of the last century. For citations to the primary literature regarding the techniques used, see the study by Rutledge and Gupta.[46]

Human Test Subjects

Testing of repellents intended for human use on human test subjects is the method of choice as it uses the repellent's end user in the testing process and can yield results relevant to actual conditions of use. The use of in vitro test methods or animal models may inadequately simulate the conditions under which repellents for use on humans are expected to perform. Tests on human subjects are carried out on adult volunteers, who may be selected from among candidates exhibiting mild or no sensitivity to arthropod bites. Equal numbers of male and female test volunteers are preferred.

Given that various factors may alter a person's attractiveness to test arthropods and that this may, in turn, affect the outcome of repellent tests, volunteers should avoid the use of fragrance and repellent products 12 hours before and during testing. Volunteers should preferably not be tobacco users or at least should not use tobacco for 12 hours before and during testing. In preparation for laboratory or field studies, the test area of the volunteer's skin should be washed with unscented soap, rinsed with water or a solution of 70% ethanol or isopropanol in water, and dried with a towel.

Laboratory Tests

The objective of a laboratory test is to estimate the effective dose of the repellent and/or the protection time provided by the repellent after application on the skin. The specific aims of the test are (1) to estimate the dose–response line and the doses of the repellent providing 50% effectiveness (ED_{50}) and 99% effectiveness (ED_{99}) against mosquito landing, probing, and/or biting; and (2) to estimate the complete protection time of a repellent, which is the time between the application of the repellent and first mosquito landing, probing, and/or biting. Landing, probing, and/or biting behavior signifies the end point of the repellent efficacy test. Landing, probing, and biting are not always associated, and a repellent may reduce biting activity without reducing landing or probing activity. However, landing and probing activity may be important in interpreting the results of the test.

N,*N*-diethyl-3-methylbenzamide (deet) is the active ingredient of most commercially available repellents and is recommended as a positive control or material standard (usually 20% in ethanol) against which the effectiveness of alternative mosquito repellents is judged.

Standardized mosquito rearing and laboratory conditions are essential to ensure the reliability and reproducibility of data. As an example, mosquitoes should be reared, maintained, and tested (in a separate room) at 27°C ± 2°C and ≥80 ± 10% RH, with a 12:12 (light:dark) hour photoperiod. Temperate and subarctic mosquito species may require modified rearing conditions. Test populations of mosquitoes should have access to sugar solution but should not be blood fed. Repellent tests should be conducted using female mosquitoes that have been starved for the preceding 12 hours and, where practical, tested during times in the diel period at which the biting activity of that species occurs.

Mosquito repellency tests should be conducted with three of the more anthropophilic *Aedes* (preferably, *Aedes aegypti*), *Culex* (preferably, *C. quinquefasciatus*), and *Anopheles* (preferably, *Anopheles stephensi*, *Anopheles gambiae*, or *Anopheles albimanus*) species. The test species, strain, and age should be reported. Mosquitoes for testing should be maintained in a stock population cage in which both sexes have been maintained to allow mating. They should be females of uniform age, preferably 5–7 days after emergence. Use mosquitoes of different age when it is more suitable, and justify such use in the study report. Active host-seeking females should be selected using an aspirator or an appropriate airflow apparatus. Mosquitoes should be contained during testing using a 35–40 cm cubical cage with a metal frame for ease of decontamination, a solid bottom and top, screening or netting on the back, clear acrylic sides for viewing, and a fabric sleeve on the front for access.

Field Tests

The objective of field trials is to extend the results of laboratory testing to estimate the effective dose and persistence of the test material against one or more mosquito vectors and/or pest species in differing ecological and/or geographical settings. A minimum of two field tests are recommended, one each in different ecological and geographical settings suitable for the target mosquito species in places where human exposure occurs. Volunteers should be obtained from the same setting in which the test is conducted so that they are not exposed to unusual risks of infection and should be protected by chemoprophylaxis and/or vaccination, if appropriate and applicable. Where possible, a site should be chosen such that there is an abundance of the target mosquito species but no ongoing disease transmission.

In the late 1950s, the efficacy of deet was evaluated using human volunteers in nipah palm–mangrove swamps on the coast of Malaysia in the vicinity of Klang, Selangor state, an environment in which approximately 30 species of mosquitoes were known to bite humans and mosquito attack rates were as high as 350 bites per man per hour.[50] These tests, conducted by scientists of the U. S. Army Medical Research Unit (Malaysia) and the Institute for Medical Research, Ministry of Health, Malaysia, were excellent examples of early repellent field tests.

Test sites: Five replications were executed in swamps along the Klang River by the Connaught Bridge. The Klang River site was selected as a representative of the most rigorous conditions for testing in Malaysia inasmuch as all previously known repellents had failed to provide protection against the hordes of mosquitoes in that area. Three other trials were carried out at Rantau Panjang, 11 km north of Klang, where about 50 species of human-biting mosquitoes and attack rates of 100–200 bites per man per hour were recorded. The diversity of mosquitoes found in abundance in the Rantau Panjang area provided an opportunity to ascertain whether the repellent might be significantly less effective against some species of mosquitoes. In each area, *Aedes butleri* and *Aedes amesi* together accounted for 60%–90% of all mosquitoes collected. Other species of *Aedes* and species of *Culex*, *Armigeres*, *Lophoceratomyia*, and *Anopheles* were also present and were at times

common. The tests were performed in late July and early August in 1958 during a season when there was some rainfall nearly every day. No rains occurred during the actual periods of exposure in the experiments reported.

Evaluation: The numbers of mosquitoes caught by volunteers on the exposed portions of their bodies after having applied deet to those areas were compared with the numbers simultaneously caught by other volunteers who had similarly used the standard U. S. Army repellent M-2020 and the numbers simultaneously caught by other volunteers who did not use repellents. The number of mosquitoes caught was used for comparison in the tests rather than the number of bites obtained per collector or per limb, as frequently used in testing mosquito repellents, because of (1) potential confusion between mosquito bites and other bites by midges, leeches, and other pests; (2) the variation in pigmentation among the volunteers, which made it easier to locate bites on the skin of some volunteers than others; and (3) the inability of most collectors to endure the bites of the myriads of mosquitoes in the mangrove swamps long enough to make a biting test valid.

Duration of efficacy: It was known from earlier laboratory and field work that deet was effective against Malaysian mosquitoes for at least 5 hours, whereas M-2020 was effective for no more than 2 hours. Accordingly, the experiment was planned to obtain data for several hours beyond these critical times. Deet was tested in intervals of 0.5, 1, 2, 3, 4, 5, 7, and 9 hours after application, and M-2020 was tested at 0.5, 1, 2, 3, 4, and 5 hours after application. Because the number of mosquitoes in the nipah palm–mangrove swamps varied considerably between areas only 3 m apart that seemed ecologically identical, the 48 collectors were divided into three groups of 16 each, each group containing equal number of volunteers with the same treatments and treatment times. Thus, each group contained eight volunteers treated with deet 0.5–9 hours before exposure, six volunteers treated with M-2020 repellent 0.5–5 hours before exposure, and two untreated volunteers. Each group of 16 volunteers sat together in a circle 15 m away from the other groups. In addition, each group moved as a unit to a new location 9–30 m away twice or thrice during the 1-hour exposure period.

Test subjects/volunteers: The volunteers participating in the experiment were experienced mosquito collectors, having been employed in that capacity at one time or another by the U. S. Army Medical Research Unit (Malaysia) or the Institute for Medical Research, Ministry of Health, Malaysia. Each had repeatedly practiced the procedure, and several pilot experiments were performed to ensure that the volunteers could perform their duties well. Certain individuals were found to be particularly attractive to mosquitoes or adept at catching them, and others were found to be inept at catching mosquitoes; the latter were excluded from the experiment to avoid bias in the results of the experiment. All Malaysian national groups were represented in the 48 volunteers ultimately selected, including Malays, Chinese, Indians, Eurasians, and Filipinos. The volunteers wore clothing ordinary and common in the tropics, that is, a short-sleeved shirt and shorts with the legs bare.

Repellents: The repellents were applied under supervision at the rate of two or three drops per limb or head of 75% deet or M-2020 repellent. The drops were shaken into the palms of the volunteers, and the hands were rubbed together and over the exposed parts of the body. Care was taken to achieve complete coverage because it is known that mosquitoes readily find and bite untreated skin patches.

Mosquito collection and identification: The mosquitoes were collected by placing a 55 × 17 mm, flat-bottomed, cylindrical glass tube over the insect as soon as it alighted and then plugging the aperture with cotton. Each volunteer collected from himself or herself only, and collections were made from the head, legs, and forearms only. Collection tubes were placed in the volunteer's own marked bag for subsequent tally and identification of the mosquitoes collected. Practiced collectors could collect as many as 350 mosquitoes per hour in this way. The mosquitoes collected by the volunteers were counted and identified in the laboratory the following day.

Experimental Design and Data Analysis

Historically, the development of experimental design and data analysis in repellent research and development has proceeded in parallel with the development of those topics in statistical mathematics. Initially, the reporting of raw data gave way to the reporting of means, ranges, percentages, and other descriptive statistics. With the advent of the analysis of variance, the *t* test for paired observations and the one-way and two-way analyses of variance came into use. This trend, in conjunction with the advent of the electronic computer, eventually led to the variety of sophisticated statistical methods in use today. In the formative years of technique, experimental design, and data analysis as applied in the research and development of insect repellents, erroneous data and errors of interpretation or analysis of data sometimes appeared in the scientific literature. Some examples are documented by Rutledge.[56]

Several aspects of experimental design and data analysis have been particularly influential in the history of insect-repellent test methods. As comparisons of two treatments under identical conditions can be accomplished most naturally and conveniently on the two forearms of a human test subject, the method of paired observations was the first formal experimental design to be adopted in repellent research. As described in detail in the section "Early Modern Period of Insect-Repellent Testing," this technique was pioneered by Bacot and Talbot[23] in 1926. It has been widely used since then and was the method of choice for many researchers, including Rudolfs,[51] King,[53] and Schreck.[27] It may also be pointed out that pairing is the basic element of the balanced incomplete block design as practiced in tests of insect repellents (see second paragraph that follows).

The concept of "protection time" (now often referred to as "complete protection time") was introduced in repellent testing by Rudolfs[51] in 1926. Protection time can be defined as the duration of the period between the application of the repellent treatment and the occurrence of the first bite, or the second ("confirmed") bite, subsequently received from a member of the insect test population. Analytically, this experimental design represents an extreme truncation of the insect test population distribution,[52] because the responses of only one or two members of the insect test population are recorded, whereas the responses of all other members of the insect test population are neglected. Historically, this truncation of the sample population was not recognized and the recorded protection times were regarded as standard normal variables.

The balanced incomplete block design was introduced in repellent testing by F. A. Morton in 1945.[54] This is an efficient but inflexible experimental design for which only a limited number of combinatorial solutions are available.[55] Only two of these combinatorial solutions, those for four and six treatments, were used in repellent tests, and the numbers of test subjects required were three and five, respectively. In practice, results were reported as adjusted mean protection times calculated by a formula that was never validated mathematically, statistically, or scientifically.[56] The adjusted mean protection times reported included negative values and others that fell outside the observed range of protection times. These nonsense values obviously invalidate the formula used in the computations and the adjusted treatment means computed. It should be noted, however, that statements of significance of differences among treatment means were not affected by the use of the erroneous formula and should be considered valid. In addition, some authors reported observed values and/or descriptive statistics such as percentages and range, which are also valid data.

Biological assay is undoubtedly the most widely used experimental design in the biomedical sciences. Besides its extensive application in vertebrate toxicology,[57] biological assay is used in insect toxicology,[58] ecotoxicology,[59] physiology,[29,60] pharmacology,[61,63] immunology,[62] sensory studies,[29,63] the social sciences,[63] analytical chemistry,[64] and other disciplines. Biological assay (bioassay) designs were introduced in repellent testing by D. J. Finney[65] in 1943. Biological assay designs for testing insect repellents have been demonstrated in a number of in vitro,[43,44] animal,[46–48]

and human[66] test systems. To date, biological assay test systems have been applied to tests of repellents against various species of chigger mites, argasid ticks, ixodid ticks, reduviid bugs, sand flies, mosquitoes, tsetse flies, and fleas.

In a biological assay, the potency of a test material is determined by observing the responses of the test species to graded doses of the test material. In general, graded doses of the test material and a null treatment (control) are applied to standardized treatment areas on an in vitro apparatus or on the skin of an animal or a human test subject. A standardized population of the insect test species is then exposed to each treatment area, and the number landing, probing, or feeding within a standardized test period is recorded. Each treatment area may be exposed to a separate insect test population (no choice test system), or all treatment areas may be exposed to a single insect test population (free choice test system). The test cage containing the insect test population may be applied externally to the treatment area, or the treatment area may be exposed inside the test cage containing the insect test population. In either case, areas of skin outside the treatment area (the hand and upper arm in a forearm test) are protected from landing, probing, or feeding. The test data obtained are typically analyzed as the linear regression of the response of the test insect population in probits (probability units) on the logarithm of the dose of the test material applied and reported as the ED_{50} (median effective dose) and the ED_{95} (95% effective dose) with their associated 95% confidence limits. The unit of measurement in bioassays of topical repellents is typically milligrams per square centimeter.

CONCLUSIONS

It has been said that "history is philosophy teaching by examples."[67] History is a resource for progress and advancement. The history of insect repellents, like the history of any science, is a record of simplicity and genius, blindness and insight, error and correction, advancement and setback, and failure and success. The researcher who is familiar with the history of his or her science is better equipped to succeed in it than one who knows only its current state. The old issues of our journals are as illuminating as the new ones.

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CHAPTER 9

Plant-Based Insect Repellents

Sarah J. Moore

CONTENTS

USE OF PLANTS THROUGHOUT THE GLOBE

Plants were first recorded being used against biting insects by the ancient Greeks,[1] and are still used by enormous numbers of people today. Most households in the developing world rely on personal protection measures of limited effectiveness, such as burning leaves,[2] despite the wide range of modern, effective malaria-control measures available, because they prefer to use an intervention that is free and widely available, despite its lower efficacy and undesirable characteristics such as strong odor.[3]

Mosquito Coils

It is estimated that 45–50 billion mosquito coils are used annually by approximately 2 billion people worldwide,[4] mainly in Southeast Asia, but with a growing market in South America and Africa. Mosquito coils were traditionally made with finely ground pyrethrum daisy (*Chrysanthemum cinerariaefolium*) flowers mixed with coconut husks or sawdust.[5] Synthetic pyrethroids, based on the molecular structure of the pyrethrins contained in the pyrethrum daisy, have outstripped natural pyrethrins for use in household pesticides[6] because they are far more photo stable, although both chemical groups possess rapid insecticidal and repellent action.[7] Nonetheless, 17,000 tons of natural pyrethrum are produced in Kenya, Tanzania, Rwanda, and Australia annually to supply the household insecticide market. There is ample evidence that mosquito coils made from both natural pyrethrins and synthetic pyrethroids effectively repel mosquitoes.[8]

Mosquito coils are made from base materials impregnated with pyrethrum or synthetic pyrethroids, which is released through slow, steady combustion. The insecticide is not known to be harmful to humans, but the smoke produced from combustion of coils is a nuisance to people, and some products generate products of incomplete combustion, which are harmful to humans.[9] It is therefore desirable to develop new means of volatilizing insecticides that are less harmful, such as passive emanation through the use of large surface area.[10] More wealthy individuals in less-developed and middle-income countries overcome this problem by using heated mats and vaporizers and mosquito coils accounting for $1.6 and $1 billion of the $8.4-billion consumer market, respectively, although electricity is required to operate them.[4,11]

Natural Fumigants

Data from economic studies indicate that although many rural households in countries of low economic quintiles do spend a substantial portion of their household income on mosquito coils (Table 9.1), they use traditional fumigants to supplement government control programs or when they are traveling away from home. Studies from Southeast Asia commonly report such practices, including 25% of mobile populations interviewed in Thailand and Cambodia,[12] 32% of households in rural Myanmar,[13] and 17% of households in southwestern China.[14] In Sri Lanka, 69% of families burned neem kernels and leaves (*Azadirachta indica*) to repel mosquitoes, along with mosquito coils (54%), despite almost all houses being regularly sprayed with residual insecticide.[15] Even so, the cost of personal protection methods is a particularly important issue, and around 2% of household income may be spent on personal protection measures (Table 9.1). The use of shop-bought preventive measures is generally higher among those of a higher economic status. In Malawi, a greater percentage of those of low to high income uses preventative measures, including coils (67% and 16%, respectively) and repellents (11% and 1%, respectively), against mosquito bites than those of very low income.[16] In India, it was noted that expenditure was linearly related to household income with those having the most income, spending the most on personal protection.[17] However, another study from India showed that community education can enhance uptake and use of personal protection, although the decision to use such tools is more related to mosquito nuisance than disease prevention,[18] as also seen in Tanzania.[19]

Table 9.1 Examples of Household Expenditure on Mosquito Bite Prevention among Those in Low Income Countries

Intervention and % Use	Location	Household Income/Month	Annual per Capita Expenditure	Proportion of Income	Ref
Coil, 61%; bed net, 57.7%; smoke, 8.3%	Rural Orissa, India	3000 Rupees or less	$4.70	2.0%	20
Coil, 79.3%; mat, 11.7%; liquid vaporizer, 11.7%	Urban Orissa, India	3000 Rupees or less	$3.00	2.8%	
Coil, 65%; liquid vaporizer, 7%; bed net 3%	Urban Chennai, India	3000 Rupees or less	97.3 rupees per month per household	3%	17
Bed nets, 0.68%; treatment of bed nets, 0.27%; repair of bed nets, 0.26%; coils, 0.15%	Rural Tanga, Northwest Tanzania	$20.88 on education and approximately $23.14 on health care per capita per year	Bed nets $0.86; treatment of bed nets $0.10; repair of bed nets $0.05; coils $0.19	<1% of monthly health budget	21
Mosquito coil, 76.8%; bed net 11.6%; vaporizing mat, 5.3%; smoke, 5.3%	Sri Lanka	61% Spent between $0.97 and $1.94 per month		3% of monthly income for those of highest income and 8% of monthly income for lowest income group	22
Bed nets 74%; treatment of bed nets 21%; repair of bed nets 18%; coils 43%; indoor sprays 11%; smoke 45%	Farafenni, Gambia (rural, peri-urban and urban)	$2.86 on education, $2.54 on health care	Bed nets $0.15; treatment of bed nets $0.02; repair of bed nets $0.01; coils $0.67; indoor sprays $0.33; smoke $0.24	25% of health-care budget is on coils	23

Among poorer populations that cannot afford shop-bought personal protection methods, natural fumigants are extensively used, and less commonly, plants are hung around the home or rubbed onto the skin. A study from rural Guatemala found that >90% of households interviewed burned waste plant materials such as coconut husks to drive away mosquitoes.[24] In Mexico this is 69%,[25] and in Colombia 50% of people reported that they burned wet logs in metal pots to prevent mosquito nuisance, especially when fishing among the mangroves[26] as was first seen among the ancient Egyptian fishermen as described by Herodotus.[27] In areas where use of fires indoors is independent of socioeconomic status, wood smoke can reduce indoor mosquito density. In a recent study conducted in Lao People's Democratic Republic, smoke from cooking fires located under the house or indoors was found to be protective against house entry by Japanese encephalitis and malaria vector mosquitoes, compared with cooking in a separate room beside the house (putative Japanese encephalitis vector incidence rate ratio [IRR] = 0.43, 95% confidence interval [CI]: 0.26–0.73, $p = .002$; anopheline IRR = 0.22, 95% CI: 0.10–0.51, $p < .001$).[28] The evidence demonstrating that biomass smoke is an effective repellent is variable, and should not be encouraged as a means of bite prevention due to the large amount of respiratory infection induced by chronic exposure to biomass.[29]

Interestingly, smoke has proven effective in preventing bites from the tsetse fly that is extremely difficult to repel even using conventional repellents such as deet and permethrin-treated clothing.[30] A study by Torr et al.[31] clearly demonstrated that smoke from burning wood (*Colophospermum mopane*) or dried cow dung reduced the catch of baited Epsilon traps by approximately 50%–90%. This study elegantly demonstrated that the smoke decreases the long-range attractiveness of the bait to tsetse flies, and also because the smoke reduced catches at unbaited traps, the smoke demonstrated

true repellency causing insects to orient away from the source rather than by attraction–inhibition. The authors suggest that the combustion products of lignin, 2-methoxyphenol and 4-methylguaiacol, and/or related chemicals may be repellent. Mosquito coils and plant-based fumigants also work over a larger area and produce smoke that may be insecticidal,[32] repellent,[31] or interfere with the perception of hosts (attraction–inhibition).[33] Mosquitoes also rely on carbon dioxide, heat, and moisture in convection currents as a short-range cue for approach to hosts.[34,35] In a field trial in Bolivia with *Mansonia titillans*, volunteers sitting close to glowing charcoal received 31% fewer mosquito landings than those sitting close to a locally bought mosquito coil (positive control).[36] However, the addition of a local plant, *Scheelea princeps*, further increased the protection to 69.2% when compared to the charcoal-only control, indicating that chemicals released from burning plants play an important role in repelling host-seeking mosquitoes. In the same way that a smoldering mosquito coil evaporates insecticide to repel mosquitoes, the insecticidal and repellent volatiles contained in certain plants may be released when those plants are smoldered or heated, which can repel mosquitoes. An innovative study from Ethiopia demonstrated that volatiles in the smoke of burning as well as fresh leaves of *Corymbia citriodora* and *Ocimum suave* have significant repellent properties against host-seeking *Anopheles arabiensis* and *Aedes (Stegomyia) aegypti* mosquitoes mainly due to the presence of β-ocimene in the headspace volatiles.[37] In the western Pacific, in Papua New Guinea, coconut husks, ginger, and betel nut leaves are burned in the early evening by up to 90% of the population and was shown to repel 66%–84% of the vector *Anopheles karwari* as well as nuisance mosquitoes.[38]

Existing data indicate that mosquito coils that incorporate plant parts or oils are less effective than conventional pyrethroid-treated coils. Field trials in Thailand measuring outdoor protection from mosquito bites in the early evening demonstrated that commercial coils such as transfluthrin 0.03% gave 84.5% protection and 0.03% d-allethrin gave 86.5% protection, whereas the incorporation of neem (*Azadirachta indica*) leaves gave 61.8% protection, citronella grass (*Cymbopogon nardus*) gave 71% protection, lemon eucalyptus (*Corymbia citriodora*) gave 67.6% protection, and Siam weed (*Eupatorium odoratum*) gave 58.8% protection. All of these significantly reduced mosquito landings on volunteers compared to a blank coil that reduced landings by 43% just through production of biomass smoke (the inert ingredients were wood powder, coconut shell powder, incense powder, malachite green, and sodium benzoate), although they were significantly less effective than the commercial coils.[39] The mosquito repellent efficacy of New Mountain Sandalwood Mosquito Sticks (containing 0.5% w/w essential oils), New Mountain Sandalwood Botanical Repellent (containing soybean and geranium oils), and a deet repellent was assessed in field tests in Australia against *Verrallina funerea* and *Verrallina lineata*.[40] A pair of burning Mosquito Sticks immediately upwind of the subject (acting as an area repellent) provided a 73.1% mean reduction in mosquito landing and probing over the 3-hour test period compared to 100% efficacy from both topical repellents.

The tradition of repelling insects by burning plants is still strongly upheld in many countries, and the popularity of repellent smoke probably lies in its convenience. As most households in the developing world use a wood-smoke cooking fire, the addition of plants requires minimum effort. Similarly, mosquito coils are the preferred antimosquito product used by low-income communities[41] because of their convenience and effectiveness.[42] For those with low household income, the utilization of waste products such as coconut husks maximizes the usefulness of a resource. However, these methods are only suitable for use outdoors because the combustion of plant materials releases many small particles and gases that have negative effects on human health.[43]

Effect of Natural Fumigants on Vector-Borne Disease Incidence

The use of traditional fumigants against mosquito nuisance in Sri Lanka was shown to be protective against malaria (relative risk = 0.58, 95% CI: 0.37–0.93), although in the same study the use of pyrethrum coils was associated with a greater relative risk of malaria (relative risk = 1.46, 95%

CI: 1.03–2.07), which the authors explain may be due to households using insufficient coils for complete protection due to cost.[44] This contrasts with findings from the Gambia showing that there was no significant difference in malaria incidence among children living in households that regularly used, or never used, smoldering *Daniellia oliveri*, although bed net use did offer some protection[45]; even though one study using human landing catches found that burning churai gave a 77% reduction (95% CI: 70–81%, t = 10.21, df = 8. $p < .001$), similar to burning a mosquito coil (71% reduction, 95% CI: 61–78%, f = 8.54, df = 8, $p < .001$).[46] A more recent study of risk factors for mosquito house entry in the same region demonstrated protective effect from burning churai of 0.56 (95% CI: 0.47–0.66) against mosquitoes entering homes.[47] In this study, almost half of the mosquito population comprised malaria vectors such as *Anopheles gambiae* s.s., *Anopheles arabiensis*, and *Anopheles melas*, whereas the other mosquitoes collected are also vectors of arboviruses and filariasis: *Culex thalassius*, *Culex quinquefasciatus*, *Aedes aegypti*, *Aedes vittatus,* and *Mansonia africanus.* In the Gambia, malaria vectors prefer to feed on human blood,[48] whereas Sri Lankan vectors will feed on cattle[49] and have the potential to be diverted to bite cattle when repellents are used. As a result of this differential vector behavior, malaria transmission is more intense in the Gambia relative to Sri Lanka.[50] The difference in the results of the two studies could also be related to erratic use of churai in the Gambia because disease prevention requires extremely high compliance with personal protection methods such as fumigants for the whole transmission season.

REPELLENT CHEMICALS IDENTIFIED IN PLANTS

Why Are Plants So Repellent to Blood-Feeding Insects?

Plants contain many chemicals termed secondary metabolites that are important in their defense against insects. These fall into several categories including repellents, feeding deterrents, toxins, and growth regulators. Most can be grouped into five major chemical categories: nitrogen compounds (primarily alkaloids), terpenoids, phenolics, proteinase inhibitors, and growth regulators. Although these compounds arose early on in plant evolution, as early as 350 million years ago,[51] as defenses against phytophagous insects, many are also effective against mosquitoes and other hematophagous Diptera.

The appearance of flowering plants in the early Cretaceous coincides with the various morphological and physiological adaptations in both insects and plants that now characterize the interdependence between insects and flowering plants. Some insect odorant receptor genes (*Or83b*, now called Orco) that facilitate the cell surface expression of odor receptors and forms heterodimers with odor receptors[52] have been conserved over 250 million years,[53] and across insect orders.[54] Interestingly, the effective repellent nepetalactone, a monoterpene obtained from the catnip plant *Nepata cateria,* was shown to be repellent to 13 widely differing insect groups including ants, caddis flies, and beetles.[55] It is postulated that floral odors developed from herbivore feeding deterrents to represent cues for mating sites and food to encourage pollination: in extant angiosperms there is almost universal occurrence of potent fragrance with chemical composition similar to many general herbivore deterrents.[56] The fact that several of these compounds are repellent to hematophagous insects could be an evolutionary relict from a plant-feeding ancestor. It has been hypothesized that blood feeding may have arisen in some insect groups, including the mosquitoes, from plant-feeding ancestors to supplement nutrition.[57] Recently, this was demonstrated under constrained conditions in a laboratory experiment where the fruit-piercing moth *Calyptrata thalictri* (Lepidoptera: Noctuidae), a subset of the males, has been found to draw blood meals from mammalian hosts. This shift in behavior has been linked to a reduction of a specific group of odorant sensory neurones (OSNs) tuned to repellent inducing vertebrate volatiles. Blood feeding could thus stem from a loss of innate repulsive behavior to vertebrate odors, leading to increased chance of zoophilic interactions

and the opportunity to feed on blood.[58] Indeed, most extant species of mosquitoes (with few exceptions) and sand flies rely on blood to provide protein only for egg development and still retain a link with plants, using nectar as a source of energy. It has now been shown that *Culex quinquefasciatus* has an odorant receptor CquiOR73 that shows strong preference to plant-derived terpenoids and phenolic compounds including the well-known plant-derived repellents *para*-menthane 3,8 diol (PMD), eucalyptol, and eugenone when expressed in a deorphanised gene in Xenopus oocytes.[59] Other research using deorphanised genes expressed in Xenopus oocytes has shown several plant-based molecules: PMD, 2-undecanone (2U), nepetalactone, and callicarpenal-inhibited AgOR8 plus AgOR7 responses to the attractant (*R*)-(−)-1-octen-3-ol in *Anopheles gambiae*.[60]

It is also possible that the fact that plant products commonly deter hematophagous insects is an evolutionary coincidence; however, it is very likely that many plant volatiles are deterrent or repellent because they have high vapor toxicity to insects.[61] In work with the mosquitoes *Anopheles culicifacies, Anopheles stephensi, Culex quinquefasciatus*, and *Aedes aegypti*, steam distillation extracts of *Tagetes erecta* (marigold) and *Mentha piperita* (peppermint) exhibited rapid knockdown activity.[62] Studies on vapor toxicity of plant volatiles to *Sitophilus oryzae* (rice weevil) showed that terpenes from the plants, including menthone and menthol, inhibit acetylcholinesterase activity.[63] This is the same mode of action as organophosphate insecticides. Several essential oil monoterpenes such as thymol, eugenol, pulegone, terpineol, and citronellal demonstrated inhibition of cytochrome P450 and glutathione S-transferase (GST) detoxification enzymes against fourth instar larvae of *Aedes aegypti*.[64]

Alkaloids

Alkaloids are insecticidal at low concentrations and are frequently toxic to vertebrates. They are nitrogenous organic molecules with varying structures. Their mode of action varies, but many affect acetylcholine receptors in the nervous system (e.g., nicotine),[65] or membrane sodium channels of nerves (e.g., veratrine/sabadilla).[66] Insecticidal examples include nicotine (*Nicotiana* spp.), anabasine (*Anabasis aphylla*), veratrine or sabadilla (*Schoenocaulon officinale*), and ryanodine (*Ryania speciosa*). Physostigmine, which served as the model compound for the development of the carbamate insecticides, is an alkaloid isolated from the Calabar bean (*Physostigma venenosum*).[67] Although these chemicals are not volatile, they may be used as repellents by burning plant material either on a fire or in a mosquito coil to create an insecticidal smoke, which repels the insects through direct toxicity. Alkaloids are found in large quantities in many members of the Berberidaceae, Fabaceae, Solanaceae, and Ranunculaceae families, all of which are used extensively as traditional insect repellents.[68,69] However, many are potent mammalian neurotoxins and their use should be limited. Tobacco is commonly used against biting insects throughout the globe,[70] although this is highly inadvisable due to the carcinogenic effects of breathing fumes from burning tobacco.

Phenols

Phenols, sometimes called phenolics, are a class of chemical compounds consisting of a hydroxyl group (–OH) attached to an aromatic hydrocarbon group. The simplest of the class is phenol (C_6H_5OH). The functions of phenols are diverse, contributing to cell wall structure, flower color, and defense against both vertebrate and invertebrate herbivores. Important phenolics in terms of insecticidal and repellent function are the flavonoids, which are characteristic compounds of higher plants. There are three important insect-repellent flavonoid groups: (1) flavones, which are found in the Labatiae, Umbelliferae, and Compositae and are quite new in evolutionary terms; (2) isoflavonoids, found mainly in the Leguminosae: an example of which is the potent mitochondrial poison rotenone[71] present in the roots and rhizomes of 60 members of the Leguminosae family, most particularly in *Derris elliptica*; and (3) tannins that are found throughout the plant

kingdom and exhibit toxicity by binding to proteins.[72] However, the large size of the phenols means that they have little significance as repellents, due to their lack of volatility and are generally phagodeterrent.[73]

Terpenoids

Terpenoids are among the most widespread and structurally diverse of the plant products with approximately 25,000 terpene structures reported,[74] all of which are derived biosynthetically from units of isoprene that has the molecular formula C_5H_8. As chains of isoprene units are built up, the resulting terpenes are classified sequentially by size as hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, and tetraterpenes. Although much information exists on their synthesis and application for cosmetic, industrial, and medicinal use, far less is known about their function in plants. There are several important groups in the triterpene category: triterpenes, steroids, saponins, sterolins, and cardiac glycosides. The widely publicized compound azadirachtin, derived from the neem tree (*Azadirachta indica*), is a triterpenoid. Azadirachtin and saponins (also found in the neem tree) are insect growth regulators (phytoecdysones). Common triterpenes include ursolic and oleanic acid, limonins, and cucurbitacins. Triterpenes are the constituents of many folk remedies, particularly in Asia as they have multiple modes of action including antibacterial and antifungal properties.[68]

Essential Oils

Monoterpenes, both cyclic and acyclic, are major components of many essential oils and are the most important group to consider in terms of repelling insects. Essential oils are complex mixtures of volatile organic compounds produced as secondary metabolites in plants, which are generally responsible for the distinctive odor of plants, and are obtained by hydro distillation, steam distillation, dry distillation, or mechanical cold pressing of plants. Their function is protection from herbivorous insects and mammals and protection from fungi[74] and bacteria.[74] Essential oils are produced in 17,500 aromatic species of higher plants belonging mostly to a few families, including the Myrtaceae, Lauraceae, Poaceae, Lamiaceae, and Asteraceae. The synthesis and accumulation of essential oils are associated with the presence of complex secretory structures such as glandular trichomes (Lamiaceae), secretory cavities (Myrtaceae, Rutaceae), and resin ducts (Asteraceae, Apiaceae).[75] Depending on the species considered, essential oils are stored in various plant organs, for example, flowers (*Chrysanthemum cinerafolis*), leaves (*Cymbopogon nardus* and *Corymbia citriodora*), wood (cedar wood, *Cedrus deodorum*), roots (vetiver grass, *Chrysopogon zizanioides*), rhizomes (ginger, *Zingiber officinale*), fruits (pepper, *Piper longum*), and seeds (nutmeg, *Myristica fragrans*).

Physiological expression of secondary metabolites of plants may be different at all stages of its development, depending on temperature,[76] circadian rhythm,[77] plant stage,[78] soil type, and climate.[79] Thus, it is important to recognize that essential oil composition may vary by cultivar, region, and even by time of harvest, and analysis of essential oil composition before testing is advisable. For commercial plant-based repellents, standardization may be achieved by using plant-derived constituents of essential oils, either produced as a product of distillation and purification, synthetically produced, or produced by biotransformation.[80]

Pyrethrins

From a public health perspective, the most important group of monoterpenes is the insecticidal pyrethrins, which are harvested from the dried heads of flowers in the *Chrysanthemum* genus, used in mosquito coils and sprays.[81] The pyrethrins are a pair of natural organic compounds that

Table 9.2 Repellency of Components Commonly Found in Essential Oils to *Stegomyia* (*Aedes*) *aegypti* Mosquitoes—1 mL per Forearm of Pure Compound

Compound	Duration of Protection (h)
Terpenene	0
Limonene	≤1
Myrcene	≤1
Eucalyptol	≤1
α-pinene	≤1
Thujone	≤1
Citronellol	1–2
Citronnelal	≤1
Eugenol	≤1
Coumarin	1–2
Linalool	1–2
Geraniol	2–3
Citral	2–3
Geranyl acetone	1–2
Thymol	1–2

Source: USDA, *Results of Screening Tests with Materials Evaluated as Insecticides, Miticides and Repellents at the Orlando, Florida Laboratory E-733*, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, Orlando, FL, 1967.

have potent insecticidal activity. Pyrethrin I and Pyrethrin II are structurally related esters with a cyclopropane core (Table 9.2). They differ by the oxidation state of one carbon. They are viscous liquids that oxidize readily to become inactivated. Pyrethrins are neurotoxins that attack the nervous systems of all insects. Pyrethrum affects the central nervous systems of all types of flying and crawling insects, blocking sodium-gated nerve junctions so that nervous impulses fail,[82] and the insect is knocked down and may die. In the lowest concentrations, pyrethrum affects insect behavior producing a loss of responsiveness to host cues, followed by increased, nondirectional activity that is roughly correlated with the concentration of the pyrethrum that results in dispersal of the organisms (orthokinesis), which is sometimes called a so-called avoidance reaction or "excitorepellency," which results in the appearance of the insect fleeing the source of the chemicals. At higher concentrations there is knock down and death.[83]

Repellent Terpenes

Many repellent terpenes present in essential oils comprise a monocyclic, carboxylic ring structure having six members and substituted by at least one oxygenated or hydroxyl functional moiety including α-terpineol, cinnamaldehyde, carvacrol, carveol, citral, citronellal, citronellol, *p*-cymene, cineole, eugenol, geraniol, menthol, piperonal, δ-pulegone, thymol, and vanillin, a property that is shared with the synthetic repellent diethyl phthalate that is commercially available in Southern Asia and protects for 4 hours in arm-in-cage tests with *Anopheles stephensi* mosquitoes.[85] Many arthropod-repellent terpenes are oxygenated, having the hydroxyl group linked to a primary, secondary, or aromatic carbon. It is important to note that for some metabolites with the hydroxyl group linked to a tertiary carbon (linalool, α-terpineol, and limonene), such activity is suppressed against *Anopheles gambiae*, suggesting the possibility that the type of carbon where the hydroxyl substitution is present modulates repellency,[86] a phenomenon also seen in *Aedes aegypti*.[87] This has been further confirmed in another study showing that benzene ring–based repellent terpenes were more repellent when they had the electronic and electrotopological properties of carbons 1 and 7 affect

repellent activity possibly because they are involved in receptor–ligand interactions.[88] These experiments found that repellency increased as the electronic accessibility of carbon 7 decreased especially due to a hydroxyl group (electron donor group) attached to a tertiary carbon resulting in strong repellency, and higher electron density around carbon 1 increased repellency. A useful development from the study of the biological activity of terpenes is the development of neural network models to investigate the relationship between biological activity, that is, duration and efficacy of repellency and molecular characteristics such as electrostatic interactions (location of poles at different parts of the molecules) giving molecules negative charge, which could influence ligand–receptor noncovalent bonds, and dipole movement that effects the shape of the molecule and can influence surface recognition between the insect receptors and repellent ligand, as well as boiling point that will affect availability of the molecule to interact with insect chemoreceptors. This is an exciting area that has already led to the discovery of a number of highly effective new repellent molecules.[89] The repellent protection times of several naturally occurring terpenes for *Aedes aegypti* in the laboratory are listed in Table 9.2.

Citronellal (CAS 106-23-0) is the main component in the mixture of terpenoids that give citronella oil its distinctive lemon scent. It is abundant in *Corymbia citriodora*, the lemon-scented gum, as well as constituting around 30% of essential oils extracted from *Cymbopogon winterinus* (Java citronella).[90] Compared to deet, a higher dose of citronellal is required to achieve repellency.[91] A 30% extract was 80% repellent to mosquitoes measured by standard cage tests with *Culex pipiens pallens* as well as when 40 volunteers were asked to record mosquito landings under normal user conditions, but mosquitoes were not collected and the method was subject to recall bias.[74] Citronellal has reported space-repellent properties against *Aedes (Stegomyia) albopictus* (article in Chinese).[92] Recent work in the Drosophila olfactory model has demonstrated that olfactory coreceptor *OR83b* contributes to citronellal repulsion and is essential for citronellal-evoked action potentials.[93] Intriguingly, this research also discovered that citronellal interacted with the olfactory coreceptor Orco (also called *OR83b*) in *Anopheles gambiae*, which is necessary to produce citronellal-induced action potentials, and citronellal directly activated action potential in TRPA1 in *Anopheles gambiae.* This is of great interest as it demonstrates a potentially new target site for insect repellents outside Orco and odor receptor ligands.

Limonene (CAS 5989-27-5) is a clear, colorless liquid at room temperatures with an extremely strong smell of oranges. Limonene is a chiral molecule, and as is common with such forms, biological sources produce one specific enantiomer. The principal industrial source, citrus fruit, contains D-limonene ((+)-limonene), which is the (*R*)-enantiomer. Racemic limonene is known as dipentene. As the main door constituent of citrus (Rutaceae), D-limonene is used in food manufacturing as a flavoring, and added to cleaning products such as hand cleansers to give a lemon-orange fragrance. However, the (*R*)-enantiomer is also used as a botanical insecticide. Limonene is found in a huge range of plants including many that are used as repellents, such as *Thymus vulgaris* (thyme), *Salvia officinalis* (sage), *Curcuma longa* (turmeric), *Acorus calamus* (sweet flag), *Corymbia citriodora* (lemon-scented gum), *Melaleuca alternifolia* (tea tree), *Ocimum basilicum* (basil), and several species of mint (*Mentha* spp.).[94] It is mentioned as a repellent constituent of 5% of mosquito patent interventions, as well as being cited as a synergist of pyrethrum,[95] despite limited longevity of <1 hour[96,97] or equal to an hour when formulated in a carrier such as olive oil or Vaseline.[98]

Myrcene or β-myrcene (CAS 123-35-3) is an olefinic monoterpene. It is obtained from the essential oil of the plants bay (*Laurus nobilis*), verbena (*Lippia citriodora*), and myrcia (*Myrcia gale*) (from which it gets its name) and others, although it can be obtained synthetically by the pyrolysis of pinene. Myrcene is one of the most significant chemicals used in the perfumery industry because of its pleasant herbaceous odor, but it is mainly used as an intermediate for the preparation of flavor and fragrance chemicals such as menthol, citral, citronellol, citronellal, geraniol, nerol, and linalool. It is also repellent to mosquitoes (Table 9.2), and is found in many plants used in both traditional and commercial repellent preparations (e.g., *Pelargonium graveolens* [rose geranium],

Melissa officinalis [lemon balm], *Hyptis suaveolens* [wild hops], *Ocimum kilimandscharicum* [African basil], *Mentha piperita* [peppermint], and *Cymbopogon nardus* [citronella]).[94] Myrcene is only mildly repellent,[97,99] although one paper reports it as repellent despite the uncertainty around their estimates as demonstrated by large confidence intervals and lack of information on replication used in the experiment.[100] An olfactometer study demonstrated similar repellency to deet at a dose of 400 µL with 2 mL/s CO_2 as the attractant against *Aedes aegypti*, but lower attraction–inhibition.[101] In a different olfactometer experiment, myrcene applied to skin at 1.4 mg/cm^2 using a human hand as the stimulus showed 80% repellence to *Aedes aegypti* among those mosquitoes that responded to either port, which was the same as seen with deet in the same experiment.[102]

Pinene is a bicylic monoterpene with two forms of stereoisomers: (1) α-pinene consisting of (1R)-(+)-α-pinene (CAS 7785-70-8), (1S)-(–)-α-pinene (CAS 7785-26-4); and (2) (1S)-(–)-β-pinene (CAS 18172-67-3) and (1R)-(+)-β-pinene (CAS 19902-08-0). As the name suggests, both forms are important constituents of pine resin; they are also found in the resins of many other conifers, and more widely in other plants including sage, *Mentha piperata* (peppermint), and *Corymbia globulus* (blue gum).[94] Less research is available on the effect of pinene as a repellent than many of the other terpenes. In an olfactometer experiment, β-pinene applied to skin at 1.4 mg/cm^2 using a human hand as the stimulus showed 60% repellence to *Aedes aegypti* among those mosquitoes that responded to either port, which was lower than recorded with deet or other terpenes in the same experiment.[102] In a recent bioassay using *Aedes albopictus* in a standard arm-in-cage method,[103] (+)-β-pinene at 0.4 µL cm^2 provided total mosquito protection, but (–)-β-pinene provided poor protection demonstrating the importance of isomers in odor receptor specificity.[104]

Citronellol or dihydrogeraniol (CAS 106-22-9), is a natural acyclic monoterpenoid. Both enantiomers occur in nature. (+)-Citronellol, which is found in citronella oils, is the more common isomer. Citronellol is found in the oils of many aromatic plants including *Pelargonium graveolens* (rose geranium), *Cymbopogon nardus* (citronella), *Mentha pulegium* (European pennyroyal), *Citrus reticulata* (tangerine), and *Melissa officinalis* (lemon balm).[94] Its characteristic sweet lemon scent lends it to many uses in the perfume industry, although it shows excellent repellency to mosquitoes (Table 9.2). In an experiment with *Aedes aegypti,* 32 and 72 mg citronellol evaporated using a standard vaporizing mat in a 60 cm × 60 cm × 60 cm chamber repelled >70% of mosquitoes with low toxicity, which exceeded that of 10% allethrin mat, although allethrin mats have a more toxic mode of action, killing 80% of mosquitoes.[105] In olfactometer studies, citronellol showed strong repellency and attraction–inhibition against the stable fly *Stomoxys calcitrans*.[106] In a thorough series of experiments in search of candidate area repellents it was shown that 0.1 citronellol has a good area repellent effect of 84% against *Aedes aegypti* using a mouse host, although this was lower than citronellal (96%) and geraniol (90%).[107] Citronellol is also highly repellent to ticks: nymphal lone star tick *Amblyomma americanum*.[108]

Eugenol (CAS 97-53-0) is an allyl chain–substituted guaiacol, that is, 2-methoxy-4-(2-propenyl) phenol. It is a clear to pale yellow oily liquid extracted from certain essential oils especially from clove oil and cinnamon. It is slightly soluble in water and soluble in organic solvents. It has a pleasant, spicy, clove-like taste and odor useful in perfumeries, flavorings, and essential oils, as well as in medicine as a local antiseptic and anesthetic. It is found in a range of spicy, aromatic plants including *Syzygium aromaticum* (clove), *Alpinia galanga* (greater galangal), *Ocimum basilicum* (basil), *Pimenta dioica* (allspice), *Cinnamomum verum* (Ceylon cinnamon), *Ocimum gratissimum* (shrubby basil), *Ocimum sanctum* (holy basil, Tulsi), *Curcuma longa* (turmeric), *Ocimum kilimandscharicum* (African blue basil), *Laurus nobilis* (bay), and *Alpinia officinarum* (Chinese ginger, lesser galangal).[94] It is highly repellent to nymphal *Ixodes ricinis* ticks with 98% repellency after 8 hours, but was only 67% repellent to *Aedes aegypti* mosquitoes after 4 hours.[109]

Linalool (CAS 78-70-6) is a terpene alcohol with many commercial applications, the majority of which are based on its pleasant scent (floral, with a touch of spiciness). It is found in many flowers and spice plants as well as in several members of the Lamiaceae including *Ocimum basilicum*

(basil), *Ocimum americanum* (American basil),[94] and *Ocimum forskolei*.[110] It has shown promise as a repellent giving 90% protection against *Culex pipiens pallens* for 1 hour at a concentration of 2% (gram per square centimeter not stated) in a choice assay[111] and evoked strong response in electroantennogram studies with *Aedes aegypti*.[110] Several studies of linalool as a space repellent, where it is continuously evaporated to protect a space, have shown a protective effect of 71% and 55% for mosquitoes and sand flies, respectively, with 5% linalool candles indoors under low biting pressures of 2 mosquitoes and 3 sand flies per man-hour, species not stated.[112] Better results were obtained with diffusers that expelled 0.1 g of linalool per hour and repelled 93% of *Aedes aegypti* indoors.[113] This result is unsurprising as linalool has been identified as a good spatial repellent[114] and highlights the importance of delivery for spatial repellents—in this case a constant high concentration of linalool reduced mosquito landings even though the number of landings on the control was very high—100 per test.

Geraniol (CAS 106-24-1), also called rhodinol, is an oxygenated monoterpene alcohol that is much favored in the perfume industry because of its pleasant warm rose-like odor. It is the primary part of oil-of-rose and palmarosa oil (*Cymbopogon martinii*) as well as many of the *Cymbopogon* genus.[90] Although it is modestly repellent when applied to the skin with an average of 3 hours protection against mosquitoes—15% deet protected for 7 hours in the same test,[115] it is strongly repellent to nymphal lone star ticks *Amblyomma americanum*.[108] Geraniol is an excellent spatial repellent that causes rapid knock down and mortality at 25%,[116] as well as high 97% protection from 0.1 g/h release diffusers and 88% protection from 0.11 g/h release candles indoors against high densities (100 landings in 3 hours in the control) of *Aedes aegypti* mosquitoes in well-designed field experiments.[113] Candles provide a useful means of diffusing spatial repellents for people in lower income brackets. For those with more money, a timed-release 0.3% geraniol dispenser showed >90% reduction in mosquito landings from *Culex pipiens* and *Aedes aegypti* over a 10 m^2 area.[117] The disadvantage of this method of diffusing repellents is the cost of the replacement cartridges that is far greater than the cost of the essential oil.

Coumarin (CAS 91-64-5) is used in 90% of perfumes as a base note and fixative as well as for its "new mown hay scent." Coumarin occurs naturally in cinnamon *Cinnamomum cassia,* which is >90% repellent for an hour to *Aedes aegypti* at a dose of 0.1 mg/cm^2 methanol extract,[118] and Tonka bean *Dipteryx odorata,* an extremely good insect repellent with 8 hour complete protection measured against *Aedes aegypti* mosquitoes[109] and in the U.S. Department of Agriculture (USDA) when applied onto a stocking, coumarin provided protection for >10 days when applied to cloth at a rate of 0.0035 g/cm^2.[84] The study by Tunon et al. used 0.0012 g/cm^2 of coumarin applied direct to the skin. Against *I. ricinus* nymphs coumarin provided almost complete protection for 8 hours[109] and it also has a spatial-repellent activity, repelling 90% of *Aedes aegypti* at a concentration of 0.001–0.003 μmol/L of air.[119]

Thymol (CAS 89-83-8) is a phenolic monoterpene that gives Thyme (*Thymus vulgaris*) and other species of this genus as well as *Ocimum gratissimum* (clove basil) their distinct odor, flavor, and antiseptic properties. It was first used as an insect repellent by the Italian army at the turn of the last century.[120] A recent article demonstrated that it was effective in repelling *Aedes albopictus* mosquitoes for 30 minutes, showing similar repellency as an equal (0.05 μL/cm^2) quantity of citronella oil when unformulated, but formulation with vanillin at 2× the concentration of thymol significantly improved longevity to 150 minutes.[99] A second study demonstrated a protection time of 1 hour against *Culex pipiens pallens* for 2% thymol.[111] Thymol has also been tested as a spatial repellent and demonstrated good efficacy against *Anopheles stephensi* when applied to a mat and gently heated using a mosquito mat vaporizer with a 15-minute exposure in a 60 cm × 60 cm × 60 cm chamber following the protocol of Tripathy et al. 2004.[121] Data demonstrated vapor toxicity (LD_{99} 203.41 mg/mat), complete repellency for an hour after the mat was used at 25.0 mg/mat.[122]

Citral (CAS 5392-40-5) comprises a pair of aldehyde terpenoid isomers based on the position of double bonds. The *trans* isomer is known as geranial or citral A. The *cis* isomer is known as neral

or citral B. Citral has a lemon-citrus odor and is commonly used in the perfume industry, although it is also effective as an insect repellent. Against *Aedes albopictus,* citral at 0.2 μL/cm^2 gave complete protection, performing also as an equivalent dose of deet, although longevity was not assessed.[104] Against nymphal lone star ticks (*Amblyomma americanum*) citral is an excellent repellent[108] and against *Aedes aegypti* a 15% solution of citral gave >85% protection for an hour using a bird host.[123] When exposed to 0.05 μg/cm^3 citral vapor for 24 hours, *Aedes albopictus* showed reductions in activation and host seeking that persisted for 72 hours after exposure[33] indicating a prolonged interaction with the mosquito's central nervous system possibly through competitive inhibition of odor receptor binding sites, prolonged depolarization of odor receptor neurons,[60] or sublethal incapacitation of the insect.[124] Application of 80 mg of citral to a vaporizing disk induced 76% repellency in *Aedes aegypti*.[125] Citral is the major constituent of the oil of lemongrass and several other members of the *Cymbopogon* genus and several citrus plants including *Z. officinale* (ginger), *Ocimum basilicum* (basil), *Cymbopogon flexuosus* (East Indian lemongrass), *Cymbopogon citratus* (lemongrass), *Aloysia citriodora* (lemon verbena), *Citrus limon* (lemon), *Mentha rotundifolia* (apple mint), and *Cymbopogon winterianus* (Java citronella).[94]

Geranyl acetone (CAS 689-67-8) is a monoterpene ketone found in with a fresh, rose, magnolia-type odor that is used as a flavoring agent and is also found in the odor profiles of people that are less attractive to mosquitoes.[126] Geranyl acetone was identified as a constituent of *Suregada zanzibariensis* Verdc. (Angiospermae: Euphobiaceae) used as a repellent by people of the coastal region of Tanzania, and in standard arm-in-cage tests with *Anopheles gambiae* mosquitoes, the dose of inhibited 50% of biting (RC50) was 49.0 mg/cm^2 × 10.4 mg/cm^2.[127] Other behavioral assays with *Anopheles gambiae* and *Culex quinquefasciatus* have shown 100% repellency and 73% repellency against *Aedes aegypti* at 10% concentration.[128] Geranyl acetone is extremely repellent to nymphal lone star ticks (*Amblyomma americanum*).[108] Against *Rhipicephalus appendiculatus*, geranyl acetone at 0.1 μL repelled 90% of ticks in a climbing assay.[129] The geranyl acetone was identified as just one of several tick repellent constituents of *Gynandropsis gynandra*, a pasture shrub that is strongly repellent to ticks—they are not found within a 2-m radius of the plant in the field.[130] Other tick repellents isolated from the plant were *m*-cymene, nonanal, 1-α-terpineol, β-cyclocitral, nerol, *trans*-geraniol, carvacrol, β-ionone, *trans*-geranylacetone, and nerolidol[129] demonstrating an interesting concept: anti-tick plants for tick control in resource-poor settings.[131]

Nootkatone was identified from Alaska yellow cedar *Chamaecyparis nootkatensis* (D. Don) as strongly acaricidal[132] after screening of promising natural essential oils used by native people of the northwestern United States.[133] Nootkatone also occurs in vetiver oil (*Vetiveria zizanioides*) and grapefruit oil, which after 4 hours had a strong repellent effect against nymphal *I. scapularis* (deer tick) that was similar to that of deet in the same climbing assay.[134] Leading on from this work, a field study of unformulated nootkatone applied to coveralls at 1.0 mg (AI)/cm^2 in walking trials demonstrated that nootkatone offered complete repellency against *Amblyomma americanum* adults for 7 days and *I. scapularis* adults for 3 days,[135] demonstrating excellent potential as an alternative to deet- or permethrin-treated clothing for consumers who prefer to use natural personal protection methods after formulation.

Nepetalactone is an organic compound, first isolated in 1941 from a steam distillate of the catnip plant (*Nepeta cateria*). Nepetalactone is bicyclic monoterpenoid, that is, it is a 10-carbon compound derived from isoprene with two fused rings, a cyclopentane and a lactone that exists in two isomers, the more prevalent one is *Z*,*E*-nepetalactone (usually about 85%) and the lesser isomer is *E*,*Z*-nepetalactone (about 15%).[136] It is best known as a cat attractant, although its insect-repellent properties have been under study for many years.[55] One of the most interesting recent studies was performed by Bohbot and Dickens using deorphanised olfactory receptor genes expressed in Xenopus oocytes. They demonstrated that nepetalactone inhibits the response of *Aedes aegypti* odor receptors AaOR8 plus AaOR7 to (*R*)-(−)-1-octen-3-ol.[60]

Volatility of Terpenes and Formulation to Improve Their Longevity

Spatial Application

The USDA has long investigated means of dispersing plant volatiles without burning them.[84] In a series of olfactometer experiments, the Center for Medical, Agricultural and Veterinary Entomology (CMAVE) has again begun investigating the use of plant volatiles as spatial repellents, that is, a compound dispensed into the atmosphere of a three dimensional environmental space that induces a range of insect behaviors induced by airborne chemicals, which result in a reduction in human–vector contact. This can include knock down, interference with host detection (attraction–inhibition), or movement away from a chemical stimulus.[137] Thus, instead of application to the skin where the repellent is evaporated on the convection currents of the host, the repellent is diffused over a larger area. In olfactometer experiments the team showed the excellent spatial repellency of catnip (*Nepata cateria*), a member of the Lamiaceae. Its spatial repellency and ability to inhibit feeding were superior to deet.[138] Field bioassays using an attractant source of light and CO_2 together with a spatial repellent showed that catnip essential oils provide some spatial-repellent protection against *Anopheles punctipennis* but is not as effective against culicines.[139] In room tests, 10% catnip oil used in a push–pull system reduced human landings by 44%.[140] Other authors studied the behavioral responses of *Anopheles harrisoni* and *Aedes aegypti* to catnip and they concluded that catnip is not a toxicant but an effective repellent at concentrations higher than 2.5%.[141] Volatilization of peppermint oil *Mentha arvensis* evaporated indoors reduced *Mansonia* spp. landings and biting by 41%, although it was ineffective outdoors against *Anopheles darlingi*.[36] The development of new ways of harnessing the spatially active repellent properties of such plant-based compounds is a new and useful area of research. Other applications are candles and diffusers, although diffusers are superior, most likely because they do not oxidize any of the repellent compounds unlike candles. Data from indoor experiments with *Aedes aegypti* showed the repellency rate of citronella candles was 14% and citronella diffusers was 68%, whereas repellency of geraniol candles was 50% and diffusers was 97%.[113] In the same experiment, outdoors, against *Culex nigripalpus*, *Aedes aegypti*, *Ochlerotatus (Gymnometopa) mediovittatus*, and *Ochlerotatus (Ochlerotatus) sollicitans*, citronella diffusers placed 6 m from mosquito traps repelled female mosquitoes by 22%, linalool repelled females by 58%, and geraniol repelled females by 75%. Trap catches were significantly reduced again when diffusers were placed 3 m from the traps. A more technologically advanced means of repelling mosquitoes is by aerosolizing plant repellents.[117] The unit disperses 0.3% geraniol/water emulsion at time intervals. At 5-minute releases, the unit can reduce biting pressure by >95% over 5.5 m against *Culex pipiens* and *Aedes albopictus.*

Formulation to Improve Plant-Repellent Persistence

When *Cymbopogon nardus* essential oil was tested in the laboratory using the screened cage arm testing method against *Anopheles minimus*, *Culex quinquefasciatus*, and *Aedes aegypti*, results showed it was only protective for 2 hours.[142] The short longevity of volatile plant oils is due to their high vapor pressure and consequent rapid evaporation.[143] Therefore, simple formulations with fixatives, emulsions, and large branching molecules, following principles commonly applied in the perfumery industry can extend repellent efficacy. Mixing citronella with large branching molecules such as vanillin can successfully retard the release rates of the volatile compounds.[144]

Formulation of 20% oil solutions in the complex solvent consisting of 20% Genapol (emulsifier and solubilizer for hydrophobic active ingredients), 10% polyethylene glycol (water soluble polymer), 20% ethanol, and 50% water improved the protection time of litsea (*Litsea cubeba*), cajuput (*Melaleuca leucadendron*), niaouli (*Melaleuca quinquenervia*), violet (*Viola odorata*), and catnip

(*Nepeta cateria*), which induced a protection time of 6–8 hours and a 100% repellency against *Anopheles stephensi, Culex quinquefasciatus*, and *Aedes aegypti*, and was more effective overall than a formulation with vannilin.[145] Recently, nanotechnology has been used to increase the duration of repellency of citronella by creating encapsulated nanoemulsions. Nanoemulsions composed of citronella oil, hairy basil oil, and vetiver oil with different droplet sizes ranging from 150 to 220 nm were tested and it was shown that the release of encapsulated limonene was controlled by the diffusion mechanism from the emulsion droplet. By using high-pressure homogenization to make smaller droplets containing repellent oils, physical stability was maximized, and that prolonged mosquito protection time to 4.7 hours against *Aedes aegypti*.[146] Another method of extending the effect of citronella is by microencapsulation using gelatin-arabic gum, which prolongs its repellency up to 30 days on treated fabric stored at 22°C.[147] Encapsulated citronella oil nanoemulsion is prepared by high-pressure homogenization of 2.5% surfactant and 100% glycerol, to create stable droplets that increase the retention of the oil and slow down release that can prolong mosquito protection time.[148,149] The use of these technologies to enhance the performance of natural repellents may revolutionize the repellent market and make plant oils a more viable option for use in long-lasting repellents. However, for the time being, travelers to disease-endemic areas should not be recommended plant-based repellents.[150] But for those communities where more efficacious alternatives are not available or are prohibitively expensive, the use of plant materials to prevent mosquito bites may provide important protection from disease vectors.

Commercial Repellents Developed from Plants

There is a sustained effort to develop novel repellents driven partially by reports of deet toxicity despite its excellent safety record[151] and for consumer preference—since 1998, the number of patents using natural repellents from essential oils has almost doubled every 4 years.[95] Much of the search for new compounds has focused on plants—publications on the repellency of essential oils are increasing year on year.[86] Funding for repellent research is also available. In response to the need to protect troops stationed overseas who regularly suffer from vector-borne disease from noncompliance with deet repellents,[152] research funding support has been provided by Department of Defense's Deployed War-Fighter Protection Program for research into new repellents.[153]

As a result, several new commercial products have become available. One example is the development of the BioUD active compound, 2U, originally derived from wild tomato plants, which was identified as a repellent in the 1980s and has since been commercialized into an Environmental Protection Agency (EPA)-approved water-based emulsion product that is an effective repellent: BioUD. In arm-in-cage tests BioUD provided >80% protection for 3 and 5 hours against *Aedes aegypti* and *Aedes albopictus*, respectively (equivalent to 7% deet), and gave 95% protection in a small 5-night study in Canada with low landing pressure of 4 landings per hour.[154]

A study by Bohbot and Dickens using deorphanised odor receptor genes expressed in Xenopus oocytes have demonstrated that 2U inhibits the response of *Aedes aegypti* odor receptors AaOR8 plus AaOR7 responses to (*R*)-(–)-1-octen-3-ol and indole.[60]

BioUD (7.75% 2-undecanone) is an extremely effective tick repellent with efficacy 2–4 times greater than deet in laboratory choice tests for *Amblyomma americanum*, *Dermacentor variabilis*, and *I. scapularis*.[155] The investigators showed that 25% dilution of BioUD was more repellent than 98% deet against *Amblyomma americanum*, and extrapolated from regression analysis that the concentration of BioUD required for equivalent repellency to 98% deet was 39.5% for *Dermacentor variabilis* and 29.7% for *I. scapularis*. BioUD is also effective against ticks when tested under field conditions, applied to socks.[156] A recent field study determined the mean protection time provided by Bite Blocker BioUD to be 140 minutes against high densities of *Psorophora columbiae*, which exceeded that of 15% deet (130 minutes).[157]

PMD (CAS 42822-86-6) is an increasingly popular natural topical repellent in the current markets, as consumers are choosing natural products over traditional synthetic alternatives such as deet or icaridin. It is the most effective commercially available plant-based topical repellents and is based on Citriodiol, a waste distillate of lemon-eucalyptus essential oil (*Corymbia citriodora*). Its remarkable efficacy and durability is probably a result of PMD's lower vapor pressure, which slows down the evaporation rate of the volatile repellent molecules. Its popularity is due to its exceptional efficacy, with field studies consistently demonstrating protection equivalent to that of deet.[158] In addition, it has low acute toxicity levels making it very attractive to consumers looking for a natural repellent as an alternative to deet (oral LD_{50} = 2,408 mg/kg and dermal LD_{50} >2,000 mg/kg in rats).[159] In South America, in an area where the local malaria vector *Anopheles darlingi* bites in the early evening before the population retires to the protection of their bed nets, a randomized control trial demonstrated that the use of PMD significantly reduced the risk of contracting malaria among users by 80%.[160] PMD is the only plant-based repellent to be recommended for vector-borne disease prevention by the Centers for Disease Control (CDC)[161] and is considered to pose no risk to human health. It is available commercially in several countries in Europe, Africa, and the United States.

PMD has undergone several trials in different parts of the world. Laboratory studies by Trigg and Hill[162] showed that 30% PMD was almost as effective as deet, the most widely available synthetic repellent, against *Anopheles gambiae*. It was determined that PMD-impregnated towelettes (0.575 g) applied to the arms of human volunteers provided 90%–100% protection against mosquitoes from laboratory-reared *Anopheles arabiensis*.[163] Field studies in China showed that the protection time from *Aedes (Aediomorphus) vexans* and *Aedes albopictus* was 2 and 5.5 hours, respectively, when PMD was used in a 20%–30% glycerol and/or alcohol formulation.[164] In Tanzania, 50% PMD in isopropanol provided over 6 hours of protection from *Anopheles gambiae* and *Anopheles funestus*.[159] In the Bolivian Amazon, 30% PMD in an alcohol base provided 96.9% protection for up to 4 hours postapplication from all mosquito species, compared to 84.8% protection from 15% deet against *Anopheles darlingi*.[165]

Commercial botanical repellents are widely available and are based mainly on PMD and citronella, although several are available that use essential oils. These essential oil–based repellents generally perform significantly less well than deet. Their average repellent protection time is between 5 minutes and 2 hours,[115,166,167] a level that is not recommended for use in disease transmission areas. However, in scenarios where vector-borne pathogen risk is low, the short protection time of natural repellents may be overcome by their frequent reapplication. Of the commercial applications, Bite Blocker performed well with a mean protection time of 7.2 hours under laboratory conditions.[115] A field test showed that Bite Blocker was repellent for 3.5 hours under intensive biting pressures from *Ochlerotatus stimulans*, *Ochlerotatus canadensis, Aedes euedes*, and *Ochlerotatus fitchii*.[168] However, it is considered a third-line repellent by Health Canada, as no independent field research has been performed on this compound.[169] Repellents made with citronella, similarly, protect for 2 hours. In field tests against *Aedes* spp. in Canada, Buzz Away (5% citronella oil, plus cedar wood, eucalyptus, lemon grass, and peppermint essential oils) and Natrapel (citronella oil 10.0%) provided 92.5% and 65.6% protection, respectively, after 30 minutes. This level of protection fell to 64.3% and 32.4%, respectively, 3 hours after application.[170]

Several new insect repellents have been developed based on a piperidine skeleton, which is present in piperine, the main active chemical agent in pepper (*Piper* spp.). Piperidine is an organic compound with the molecular formula $C_5H_{11}N$. It is a cyclic amine with a six-member ring containing five carbon atoms and one nitrogen atom. It is a clear liquid with a pepper-like odor. During the 1970s, around 600 synthetic compounds related to piperidines were developed by scientists at the USDA research centers in Beltsville, Maryland and Gainesville, Florida. The data from these experiments are now being reexamined using new, recently developed methodologies coupled with rapid screening bioassays. This interest in finding deet alternatives has been motivated by

the controversy around the safety of deet, its low user acceptability, and its plasticizing effect. The repellent 1-piperidinecarboxylic acid, 2(2-hydroxyethyl)-, 1-methylpropylester was developed by BAYER in the 1980s using molecular modeling,[171] and, more recently, optically active (1S, 2S)-2-methylpiperidinyl-3-cyclohexen-1-carboxamide (SS220) has been developed as a highly effective synthetic arthropod repellent.[172] Field studies in the United States have compared the efficacy of piperidine to deet against wild mosquitoes and black flies and concluded that both compounds had similar efficacy,[173] and a randomized control trial is underway to investigate the impact of picaridin on the incidence of malaria.[174]

Citronella Group Family: Poaceae

Cymbopogon is one of the most important essential oil yielding genera of the family Poaceae that contains several plants that are used throughout the world as insect repellents. They are rapidly growing grasses with distinctive aromatic foliage. Originating in India, the group is widely cultivated throughout the tropics and subtropics in Asia, Africa, and America with a regular distribution ranging from mountains and grassland to arid zones. The most common economic species are *Cymbopogon nardus* (citronella), *Cymbopogon martinii martinii* (palmarosa), *Cymbopogon citratus* (lemongrass), and *Cymbopogon winterianus.* The essential oils from *Cymbopogon* species contain a wide variety of terpenoids, some of which like geraniol and its ester, citronellol, and citronellal are important perfume materials. Other constituents such as citral are used in vitamin A and ionone synthesis. Repellent compounds contained in this group include α-pinene, camphene, camphor, citrals (neral and geranial), citronellal, citronellol, geraniol, geranyl acetate, limonene, and terpenen-4-ol, although environmental conditions cause the content of volatile oils in plants to vary greatly.[90] Citronella grasses *Cymbopogon nardus* and *Cymbopogon winterianus* were named in two-thirds of patents lodged between 1988 and 2010.[95]

Cymbopogon nardus or citronella is the best-known member of the group, and it is used in many commercial repellent preparations. These repellents are marketed for use on children, as natural repellents are perceived to be safer for use on children than deet. Although its ED_{50} (effective dose for 50% reduction in bites) is similar to that of freshly applied pure deet,[175] its longevity is far inferior to that of deet at 2 hours.[176] A meta-analysis of 11 studies using the arm-in-cage method with *Aedes aegypti* demonstrated that citronella oil was less repellent than deet, with a difference in protection time of 253 minutes (95% CI: 169–336) and evidence (although limited) indicates that a combination of citronella oil and vanillin product has a comparable protection time to deet.[177] Citronella is also often popularly found on scented candles that are advertised as repellents. These, however, only work if the host is within 1-m radius of the candle, only slightly reduce the number of host-seeking mosquitoes[178,179] and do not reach the 50% efficacy that is required by EPA to consider the product repellent.

Cymbopogon martinii martinii (palmarosa) is a perennial grass, widely distributed throughout the tropics. It contains between 750 and 4750 ppm geraniol[94] that gives it a sweet scent. The oil of *Cymbopogon martinii* is used in traditional Indian mosquito repellent preparations.[180] Two field-tests of palmarosa against *Anopheles* mosquitoes in India showed that the pure oil provided absolute protection for 12 hours.[181,182] However, the tests utilized pairs of volunteers, one acting as bait who laid in a cot, whereas the other collected mosquitoes from him. This methodology may inflate the protection time of repellents.[183] A topical repellent commercial product containing 25% geraniol was tested in the laboratory against *Aedes albopictus, Culex nigripalpus*, and *Ochlerotatus triseriatus*, its average repellency time was a favorable 3.1 hours.[115]

Cymbopogon citratus (lemongrass) is also traditionally used as a mosquito repellent in India.[180] Evaluation using an electroantennogram showed that *Cymbopogon citratus* elicited a spike response similar to that of deet.[184] Field tests in Bolivia showed that 25% *Cymbopogon citratus* in ethanol provided 77.93% and 90.67% protection for 3 hours against *Anopheles darlingi* and *Mansonia* spp.,

respectively.[185] However, laboratory evaluation showed lower repellency at only 30 minutes complete protection.[186] A recent evaluation using video tracking showed that lemongrass oil was strongly spatially repellent to Stable flies *Stomoxys calcitrans*; although the size of the arena used to track the flies and the sample size were small, data suggest that the oil elicited an avoidance reaction through concentration mediated rate of turning (klinokinesis).[187]

Cymbopogon winterianus essential oil has been evaluated as a mixture with 5% vanillin against *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles dirus.* It compared favorably with 25% deet giving greater than 6 hours protection against all three mosquito species in cage experiments.[188] Used in South Africa as a mosquito repellent, *Cymbopogon excavatus* evaluated in the laboratory against *Anopheles arabiensis* gave good protection for 2 hours, but declined to 59.3% protection after 4 hours,[189] which compares favorably with *Cymbopogon nardus.*

Ocimum *spp. (Lamiaceae)*

The essential oils from the species of this genus contain linalool, linalol, linoleic acid, *p*-cymene, estragosl, eucalyptol, eugenol, citral, thujone, ocimene, camphor, methyl chavicol, and oleic acid, as well as many other terpenes, all of which are effective repellents.[94] It grows rapidly under a range of climatic conditions, although it is best adapted to a drier climate.

In Tanzanian tradition, fresh *Ocimum* spp., called kivumbasi, are burned, and freshly cut twigs of *Ocimum suave* and *Ocimum canum* are placed in the corners of rooms to prevent mosquitoes from entering.[190,191] The latter method was field tested in Guinea-Bissau, West Africa and fresh *Ocimum canum* (also known as *Ocimum americanum*) provided 63.6% protection from mosquito biting for 2 hours.[192] Fresh leaves of *Ocimum forskolei* are commonly hung by the side of the beds in Eritrea to repel mosquitoes, resulting in approximately 50% fewer *Anopheles arabiensis* found indoors.[193] In Zimbabwe, *Ocimum* spp. leaves are rubbed on the skin as a method of repelling mosquitoes.[194] When the juices from the leaves of *Ocimum suave* and *Ocimum canum* were spread on the legs of human volunteers, there was approximately 50% reduction in the proportion of female *Anopheles gambiae* mosquitoes that were engorged with blood.[191] A 250 mg/mL concentration of dried *Ocimum canum* leaves in ethanol provided 70% repellency against *Aedes aegypti* for 2 hours.[195] In Thailand, a 25% concentration of *Ocimum canum* essential oil in ethanol provided 3, 4, and 8 hours protection from the bites of *Aedes aegypti, Anopheles dirus,* and *Culex quinquefasciatus*, respectively, with increased protection times when used in combination with 5% vanillin.[188] Studies from Nigeria have shown good repellency of 20% *Ocimum gratissimum* in liquid paraffin (mineral oil) against *Simulium damnosum*,[196] and 30% *Ocimum gratissimum* in olive oil against *Anopheles gambiae* and *Anopheles funestus*.[197] Although these kinds of formulations are safe, cheap, and effective, their greasy feel may require some refinement to make them more acceptable to users in dusty environments.

Hyptis *spp.*

The repellent activity of *H. suaveolens* is associated with its strong smell. The chemical contents of *H. suaveolens* leaves include β-pinene, sabinene, 1,8-cineol, β-caryophyllene, (–)-sabinene, limonene, α-pinene, and bergamotene.[198,199] In the Brazilian Amazon, *Hyptis* spp. (locally called Hortelã-do-campo) are traditionally burnt and leaves are rubbed on the skin to keep mosquitoes away.[70] This method would appear effective because at very low concentrations (RD90 = 0.00048 μg/cm^2) essential oils extracted from *H. suaveolens* can repel 90% of host-seeking *Aedes albopictus* for up to 2 hours,[200] and 3 mL of 8% oil (a very high application rate) will repel >80% *Culex quinquefasciatus* for up to 6 hours.[201] At a more realistic 6.3 μg/cm^2 the steam distillate of *H. suaveolens* repelled 60% of mosquitoes in Laos, but was not effective against day biting *Armigeres* spp.[198] In West Africa the fresh plant is sometimes used, or the aerial parts

of the *H. suaveolens* are placed on charcoal and the resulting smoke repels the mosquitoes,[192] although thermal expulsion of the plant volatiles actually attracted mosquitoes.[202] In Tanzania, freshly picked and bruised sprigs of *H. suaveolens*, in local language called hangazimu, are hung in the house to try to prevent mosquitoes from entering,[203] but there was no reduction in biting when hung in an experimental hut (Curtis and Lines, 1986, unpublished). When tested in Guinea-Bissau, the fresh plant was able to provide approximately 70% protection from biting for 2 hours.[192] The smoldering plant provides the most effective protection. Nicholson and Lines (1987, unpublished) showed that there was a 10-fold reduction in biting in the presence of hangazimu smoke. Similarly, Pålsson and Jaenson[192] showed that smoldering *H. suaveolens* provided approximately 84% protection for 2 hours against *Anopheles gambiae*; whereas, Seyoum et al.[202] found only a 20.8% reduction in biting.

Thymus

The volatile compounds of *T. vulgaris* have been identified as carvacrol, *p*-cymene, linalool, c-terpenene, and thymol[111] and that of *T. magnus* as γ-terpenene, thymol, β-bisabolene, *p*-cymene, α-terpenene, myrcene, β-caryophyllene, α-thujene, camphene, carvacrol, and α-pinene.[204] Of these, carvacrol, thymol, eugenol, and carvacrol methyl ether are effective repellent compounds with minimum effective dosages in the range of 0.013–0.063 mg/cm^2.[205] Thyme oil at 100% is repellent against *Anopheles quadrimaculatus*, *Anopheles albimanus,* and *Aedes aegypti* for at least 30 minutes, when applied to cloth.[176] Different concentrations of the essential oil of red thyme were tested in the laboratory against *Aedes aegypti* and *Anopheles albimanus* with 135 and 105 minutes protection, respectively, using pure oil; and 45 minutes protection against both species at 25% concentration.[206] In the former Union of Soviet Socialist Republic, a local method against biting insects was tying thyme stick, *T. serpyllum*, with thick cotton, drying these, and then burning them. Rubtzov tested this method and reported 85%–90% protection for 60–90 minutes in the open air.[203] There may be a use for thymol as a spatial repellent as it is also insecticidal[122] and antiseptic with a pleasing odor, or as a clothing treatment because it can irritate the skin if used at high concentrations.[207]

Lantana camara (Verbenaceae)

Lantana camara (Lantana) has several important qualities that make it effective in preventing mosquito house entry. It contains a variety of terpenes and alkaloids, including high quantities of caryophylene[208] that has good repellent efficacy against *Anopheles gambiae* s.s.,[209] as well as eucalyptol, α-humulene, and germacrene that are toxic to adult mosquitoes.[210] The efficacy of Lantana as a mosquito repellent has been demonstrated by many authors. In Tanzania, a pilot efficacy study of house screening with Lantana was performed at village scale over a year.[211] Families within the study village planted Lantana around their homes and were responsible for maintaining the plants, and mosquitoes were collected using CDC light traps (CDC LT) indoors. The IRR with 95% CI relative to houses without plants in an adjusted analysis demonstrated 56% fewer *Anopheles gambiae* s.s. (IRR = 0.44, 95% CI: 0.28–0.68, $p < .0001$); 83% fewer *Anopheles funestus* s.s. (IRR = 0.17, 95% CI: 0.09–0.32, $p < .0001$), and 50% fewer mosquitoes of any kind (IRR = 0.50, 95% CI: 0.38–0.67, $p < .0001$) in houses with Lantana relative to controls. As Lantana grows wild in Tanzania, it can be provided to a household averaging five people for an initial outlay of US$1.50 making it an extremely economically attractive compliment to existing malaria-control strategies.

In a Kenyan study, Seyoum et al.[212] used 10 potted Lantana plants hung close to the eaves of 4 houses over 24 nights, and also used CDC LT as a proxy for human exposure to host-seeking mosquitoes.[213] The authors demonstrated a 27.22% (95% CI: 0.04–47.16) reduction in house entry of *Anopheles gambiae* s.l. (mainly *Anopheles arabiensis*) and no repellent efficacy against *Anopheles funestus*, contrary to the significant 83% reduction seen in Tanzania. The reason for this difference

may be related to mosquito density as the average nightly catch of anophelines in the Kenyan study was >300, whereas the Tanzanian study collected <5 anophelines per night. In addition, Seyoum et al. noted that *Anopheles funestus* in western Kenya have low sensitivity to repellents.[214] However, it is important to consider that the plants used in the Tanzanian study were over 80 cm tall and as such will have emitted a greater amount of volatile compounds than those in potted plants used in Kenya. Lantana emits very large amounts of volatile organic compounds from the leaves[215,216] including α-pinene that is a known mosquito repellent.[84] The α-pinene emission from live Lantana is almost an order of magnitude greater than that emitted from live eucalyptus and warrants further study as it may explain the ability of undamaged Lantana to repel mosquitoes (as opposed to most plants that require some mechanical damage to promote release of repellent "green volatiles").[217]

Of additional importance, there is a well-researched body of evidence indicating that mosquitoes feeding on Lantana flowers have reduced survival.[218–220] This is important because increasing mosquito access to sugar could enhance their survival and vectorial capacity by extending female life span.[221] This was recently demonstrated in a field experiment where populations of *Anopheles sergentii* with better access to sugar resources were more likely to transmit malaria. The authors demonstrated that mosquito survival was enhanced so that a greater proportion of mosquitoes survive long enough for the malaria parasite to develop, and the period between blood-feeding events was shortened, increasing the probability that they are infected or infect a human when blood feeding.[222] The availability of sugar has important epidemiological ramifications because the absence of sugar increases the number and frequency of blood feeds that are taken from man,[223] increasing vectorial capacity.[224] Thus, Lantana increases the availability of sugar to mosquitoes, but actually those mosquitoes that feed on Lantana in the laboratory have lower survival[219] and lay fewer eggs[218] than mosquitoes that feed on other sugar sources including domestic plants.[225] This negative effect on mosquito survival is a highly desirable characteristic for any vector control tool as it reduces the population size of the vector and the probability that mosquitoes will live long enough to transmit the malaria parasite. Therefore, even those not using the plant to prevent mosquitoes entering their homes may benefit from the "community effect" on malaria transmission. However, a large community-based study to measure the influence of Lantana on the mosquitoes and clinical outcomes would be necessary to measure such a potential effect.

The most important quality of repellent plants as a concept for household protection is that they are extremely cheap, widely available, and they are self-sustaining. Lantana originates from South America, but was introduced as an ornamental garden plant into Africa in the mid-nineteenth century[226] and is now naturalized in many African countries including Kenya, Uganda, and Tanzania. Lantana is extremely tolerant of drought and frost, survives up to 2000 m above sea level, and grows well without being tended. Maintenance required once the plants are established is to prune back the plants when they become too large. Therefore, the duration of protection is continuous after the plants reach sufficient height to impede mosquito entry into homes via eaves, windows, and cracks in walls. Thus, with minimal compliance, a household is provided with a means of preventing mosquito house entry that protects throughout the year on a continuous basis with resources available in the community.

For better community acceptance, it is advantageous if the repellent plant chosen has multiple benefits. Lantana is pleasing to look at and for this reason is a common garden ornamental. It is also useful as a hedge as it is dense with prickles, and planting it close to the windows can improve the security of the home. Lantana may be used as a leaf mulch to prepare the ground for crops and it improves the fertility of rocky, gravel, or hard lateritic soils, enriches the soil as the ash is rich in potassium and manganese, serves to retain humus in deforested areas, and checks soil erosion.[208] Lantana twigs and stems serve as useful fuel for cooking. However, Lantana is highly invasive and is a major weed in many regions of the Palaeotropics, in particular island ecosystems.[227] The plants can grow individually in clumps or as dense thickets, crowding out more desirable species and is a refuge for tsetse flies[228] and must, therefore, not be widely planted without a thorough ecological assessment.

Daniellia oliveri (Fabaceae)

The local names churai, santang, and santão refer to resins and wood commonly burnt indoors in western Africa to prevent mosquitoes from entering at night.[46,192,229] In several field trials, it was determined to be an effective, accepted, and cheap form of personal protection. In Guinea-Bissau, smoke from the burning bark of *D. oliveri* reduced biting from mosquitoes by 74.7% and 77.9% in comparison to the control in two separate field experiments.[192] In Banjul, the Gambia, santang reduced biting on human subjects by 77%, which was more than a permethrin mosquito coil, but less than that of deet soap.[46] A study from the Gambia[47] found churai reduced house entry of mosquitoes. Odds ratio relative to nonuser was 0.56 (95% CI: 0.47–0.66), although under high transmission no protective effect against malaria from use of churai was found.[230]

Tagetes spp. (Asteraceae)

Tagetes species contain monoterpenoid esters,[231] and their larvicidal and insecticidal activity is well-established.[232–235] Studies in Zimbabwean communities showed that people use fresh plant material of *T. minuta* as a form of personal protection by crushing the plant material and applying it to the skin, burning it, or simply exposing the whole plant.[195] Okoth[236] tested the effectiveness of whole-plant material of *T. minuta* against *Mansonia uniformis* and *Anopheles marshalli* mosquitoes in Uganda. Human landing catches were performed in a tent in which 4 kg of fresh *T. minuta* whole-plant material had been placed 1 hour previously, and in a control tent with no plants. Fewer mosquitoes were recorded biting and resting in the tent where the *T. minuta* plant material had been placed in comparison to the untreated tent. Preliminary laboratory tests also showed that the plants had repellency in a choice test, and significant toxicity when mosquitoes and plant parts were put in containers together. Tyagi et al.[237] carried out cage tests using the essential oil of *T. minuta*. After 6 hours, 86.4% protection was provided against *Anopheles stephensi*, 84.2% against *Culex quinquefasciatus*, and 75% against *Aedes aegypti*. Steam distillate of *T. minuta* evaporated at room temperature caused rapid knock down of mosquitoes including *Anopheles culicifacies* and *Anopheles stephensi*.[62] These results suggest that this plant has excellent potential as a mosquito repellent, and further testing is required. On the other hand, the essential oil extract of a sibling plant of the same genus, *T. filifolia*, was tested in the laboratory and proven not to have significant repellent effect on host-seeking *Aedes aegypti*.[238] This is because essential oil composition may vary considerably between aromatic plant species and varieties from different geographic areas due to either soil characteristics or environmental reasons.[239,240] This has proven to affect yields in essential oils from *T. minuta* plants living in soils with different humidity levels.[239]

Lippia spp. (Verbenaceae)

In the Gambia, *L. cheraliera* leaves are traditionally used as mosquito repellents. *L. javanica* is commonly found in southern Africa and is frequently used as a repellent.[195] The leaves have a strong lemon smell,[105] probably accounting for the local belief in its healing abilities. *L. cheraliera* is also burned in the Gambia as a mosquito repellent smoke.[241] A thorough study carried out in Zimbabwe revealed that 29% of the population used plants to protect themselves from mosquitoes, mainly by burning the leaves of *L. javanica*.[195] An ethnobotanic study in the Umkhanyakude district, KwaZulu-Natal, South Africa described that more than 90% of the community members burned dry *L. javanica* plants to repel mosquitoes.[242] The main constituents of the essential oils of this plant are monoterpenoids, such as myrcene, caryophyllene, linalool, *p*-cymene, and ipsdienone. An alcohol extract of dried *L. javanica* leaves was tested on human subjects against *Anopheles arabiensis* mosquitoes in the laboratory.[189] The protection was 76.7% after 4 hours and 59.3% after 5 hours. Alcohol extracts of the dried leaves applied to the skin were also shown to provide 100%

protection for 2 hours against *Aedes aegypti*.[243] Work using the related *L. uckambensis* has shown that the release of volatiles from the leaves through thermal expulsion reduces *Anopheles gambiae* biting by 49.5%.[202]

Artemisia spp.

Members of this genus are found all over the world, from tropical India to Siberia. They are low-growing perennial herbs in the family Asteraceae. The plants are aromatic, can tolerate poor conditions, and provide good cattle fodder. These plants have been used against insects for centuries. In China, bundles of dried *A. vulgaris* are burned to repel biting insects. This observation led to an investigation by Hwang et al.[102] which revealed that *A. vulgaris* contains insect repellents that can be released from the plant by combustion. The compounds isolated and found repellent to *Aedes aegypti* were camphor, linalool, terpenen-4-ol, α- and β-thujone, β-pinene, myrcene, limonene, and cineol. The plants are also burned in Central Asia, Bolivia, and India to repel mosquitoes.[70,180,203] They were burned to drive away mosquitoes by many Native Americans, including the Shuswap, Thompson, and Blackfoot tribes.[244] Extracts of *A. vulgaris* are also highly toxic to mosquito larvae.[245]

A. absinthium (absinthe) is a native of Europe, Central Asia, and Africa, yet it was only used as an insecticide in Europe[246] and India.[247] It is insecticidal[248] and contains many repellent chemicals including thujone, terpinen-4-ol, linalool, nerol, geraniol, α-pinene, and 1,8-cineole.[249] Although it is reported as a mosquito repellent,[250] *A. absinthium* does not appear to have been evaluated against mosquitoes although it is repellent to ticks.[251] Plant extracts of *A. monosperma* have been tested against host-seeking mosquitoes in the field in Egypt[252] and results show good repellency up to 4 hours. However, the plant extracts were done with petroleum ether, which on its own is repellent to mosquitoes, so results may be biased. Further research to better evaluate *A. monosperma* is necessary to draw better evidence-based conclusions.

Neem

Neem, *Azadirachta indica*, originates from India where it has been used to control and repel insects for thousands of years and is now naturalized in drier parts of Central and South America, Africa, Australia, and Southeast Asia, notably southern China where extensive plantations may now be found. Neem is widely used in its raw form as an agricultural pesticide[253] and its leaves are traditionally burned to repel mosquitoes in Africa[192] and South America,[70] whereas the leaves and husks are burned for this purpose in Sri Lanka.[15] The trees can grow in depleted and saline soil making them an excellent method of regenerating desertified or marginal land. They are fast growing and can be used for a multitude of purposes besides insect control, including firewood, fodder for livestock, and as a shade tree.

Extensive research has been carried out on the effect of botanical derivatives of the neem tree and its relatives.[254–256] *Azadirachta indica* contains at least 35 biologically active principles, of which azadirachtin is the most well-researched active ingredient. It is found in the seed, leaves, and bark. The azadirachtin content of neem oil is positively correlated with its effect against insects,[257] which may be grouped into six categories: antifeedency, growth regulation, fecundity suppression, sterilization, oviposition repellency or attraction, and changes in biological fitness.

Neem extracts have insecticidal activities against a variety of disease vectors, ranging from mosquitoes to ticks, head lice, bed bugs, cockroaches, mites, and sand flies.[258–260] The repellency of neem oil to hematophagous insects has been tested, although the results have been variable. In a well-designed laboratory assay, a commercial product containing an unspecified concentration of neem oil was tested against *Aedes albopictus, Culex nigripalpus*, and *Ochlerotatus triseriatus* demonstrating to be effective only for 1.5 hours.[115] Burning and thermal expulsion of the leaves

produce only a modest reduction (<25%) in biting.[202] Two field tests in India with *Anopheles culicifacies* using 2% neem oil in coconut oil provided 100% protection for 12 hours.[261,262] Another field test against *Anopheles darlingi* was performed in the Venezuelan Amazon using Neemos gel, a commercial preparation based on neem oil and citronella in a carbomer base. Neemos gel, offered 98.2% protection against *Anopheles darlingi* for 8 hours.[263] Although numbers of mosquitoes were high (217 anophelines per man-hour) in hand catches prior to the tests, the number of mosquitoes captured from the control was far lower at 13.78/man-hour. In each of the three tests demonstrating good efficacy, repellent or control volunteers lay on cots that reduce the number of attempted feeds that an individual receives.[46] Other individuals collected mosquitoes from the volunteers, so mosquitoes may have been diverted from repellent-treated volunteers to untreated collectors.[183]

In field tests with *Anopheles dirus*, 66.7% protection was recorded after 9 hours using 2% neem oil diluted in mustard oil again during trials with low landing rates of only 5.25 mosquitoes/man-hour on controls.[264] In contrast, when Pandian and Devi[265] tested neem oil in coconut oil against *Armigeres subalbatus*, they found that it provided only 124 minutes protection. In comparison, in the Bolivian Amazon with high densities of *Anopheles darlingi* (mean 71 mosquitoes/man-hour) 2% neem oil in ethanol provided only 56.7% protection 3 and 4 hours after application.[165] Interestingly, a field trial was conducted in India, where volunteers applied 2% neem oil in the same way as in the aforementioned trials, but sat on the ground. The protection provided by neem oil was only 73% in the first hour after application.[266] EPA has not approved neem oil for use as a topical insect repellent as it confers limited protection against mosquito bites, and therefore it is not recommended for individuals who require highly effective protection such as those traveling or living in countries where vector-borne diseases are endemic.[267]

The most effective result was obtained by vaporizing neem oil from mats: 5% neem oil was more effective at reducing both biting and numbers of resting mosquitoes than 4% allethrin on mats.[268] However, the use of electrical mats in developing countries is not an appropriate technology, because most rural households do not have electricity. It was also proposed that neem may be used to repel mosquitoes by adding it to kerosene for use in kerosene lamps used to light homes throughout the developing world.[269] Adding 1% neem oil to kerosene provided up to 84.6% protection from bites. Unfortunately, the paper is not clear as to whether treatments and control collections were carried out on the same day, neither is any mention made of baseline mosquito numbers. When 1% transfluthrin was added to kerosene only a 43.8% reduction in biting was observed.[270] If neem oil in kerosene is effective in repelling mosquitoes, this has important implications for malaria control due to the ease of application of this method. Neem oil is cheap and freely available throughout India and many other regions of the world. Perhaps a better way of releasing the volatile repellent might be to place the repellent and oil mixture above the flame and not in the kerosene itself. Transfluthrin (0.5%) volatized in this way provided >90% protection.[270] The advantage of this method was that the optimal temperature for release of the volatile repellent substance could be better regulated. Kerosene lamps are widely used so that this method offers considerable promise because of its extreme simplicity and convenience.

Garlic

It is still a common misconception that eating garlic, *Allium sativum*, will make the skin unpalatable to mosquitoes,[271] a view that has been held since ancient times.[272] Garlic is still used as a repellent in South America (hung around the neck) and China (eaten).[70,185] Stjernberg and Berglund[273] claimed that the consumption of 1200 mg garlic per day provided significant protection from tick bites. However, the accuracy of the study has been contested since the findings were exaggerated statistically due to the incorrect use of the collected data.[274] Conclusive evidence that consumption of garlic does not repel mosquitoes has been found using a double blind randomized trial.[275]

Rubbing garlic cloves on the skin does have a moderate repellent effect,[276] but is an extremely unpleasant means of protecting oneself from mosquito bites.

Lemon–Eucalyptus Extract

The lemon–eucalyptus extract comes from the plant *Corymbia citriodora* (old nomenclature includes *Eucalyptus citriodora* and *Eucalyptus maculata* var. *citriodora*) originating from China. Chemical analysis of *Corymbia citriodora* showed that it contained citronella, citronellol, geraniol, isopulegol, δ-pinene, and sesquiterpenes.[164] The essential oil extract was determined to have mosquito-repelling properties against *Aedes aegypti*, although these were limited to 1 hour.[164] This protection period is slightly superior to the essential oils of several other species of eucalyptus.[186,192,277,278] *Cymbopogon citriodora* also repels mosquitoes when the leaves are heated on a metal plate over a traditional cooking fire in western Kenya, reducing *Anopheles gambiae* landings on occupants of a house by 74.5%, which is comparable to insecticidal mosquito coils.[202]

However, PMD was discovered as a by-product. It is a white, waxy material, semisolid at room temperature, produced as a distillate after acid modification of the lemon eucalyptus oil. This material was determined to be highly repellent, and was given the Chinese name Quwenling that means "effective repeller of mosquitoes." PMD is the only plant-based repellent that has been advocated for use in disease-endemic areas by the CDC,[279] due to its proven clinical efficacy to prevent malaria[280] and is considered to pose no risk to human health.[281] It should be noted that the essential oil of lemon eucalyptus does not have EPA registration for use as an insect repellent.

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CHAPTER 10

Insect Repellents Derived from Australian Plants and Implications for Public Health Messages

Cameron E. Webb

CONTENTS

INTRODUCTION

Mosquito-borne diseases remain an ongoing concern for communities in Australia as urbanization continues to encroach on mosquito habitats and we create opportunities for mosquitoes within our urban areas. Although there is a range of options available to reduce mosquito populations[1] including a reduction in the risk of mosquito-borne diseases,[2] the first line of defense against biting mosquitoes will remain personal protection strategies.

There are approximately 5000 cases of human illness caused by the mosquito-borne Ross River virus and Barmah Forest virus every year in Australia.[1] However, it is often stated that the official statistics represent an underestimate of the total impact of these pathogens as many cases are either mild or go undiagnosed. Notwithstanding the potential public health risks of mosquito-borne disease, nuisance biting can also have severe impacts on local communities.[3] Studies have shown that the pest impacts of mosquitoes and biting midges can result in the perception that property values are significantly lower compared to suburbs with less severe biting insect problems.

To reduce the impact of nuisance-biting mosquitoes and, where possible, incidence of mosquito-borne disease, local authorities often issue health warnings that generally always contain messages of personal protection strategies. In addition to the use of insect repellents, behavioral practices (avoidance of productive mosquito habitats and peak biting times) and physical barriers (use of protective clothing, appropriate screening of housing, and bed nets) are required to reduce the risk of mosquito bites.[4,5]

Given the emphasis on personal protection strategies and the propensity of Australians to spend much of their summer outside, many of the commercial insect repellent formulations (Aerogard®, Rid®,

and Bushman™) have become iconic Australian brands. Despite the widespread use of these products, there have been few studies undertaken to investigate how individuals choose and use mosquito repellents. In one study, between 31% and 44% of Queensland residents report that topical repellents are the most common form of personal protection strategy.[6] However, despite the demonstrated safety and effectiveness of *N,N*-diethyl-3-methylbenzamide (deet) and picaridin,[7] there is growing resistance to the use of these chemical repellents by some sectors of the community. Much of the reluctance is due to perceived adverse health effects of "chemical" repellents.[8] Many also report that they are unpleasant to use and may damage clothing or belongings.[8,9] For these reasons, there is a growing demand for alternative "natural" products, particularly those derived from botanical materials.[10,11]

Repellents based on botanical extracts are often perceived to be a "natural" method to protect against mosquito bites. Native plants from throughout the world, often with links to traditional use by indigenous cultures, have been purported to have usefulness as topical repellents of biting insects.[12] A wide range of plant extracts have been tested and some products with potential benefits are derived from Australian native plants. In particular, native plants that belong to the Myrtaceae Family such as *Eucalyptus* spp., *Leptospermum* spp., and *Melaleuca* spp.

Topical repellents, regardless of their active ingredients, must be registered with the Australian Pesticides and Veterinary Medicines Authority (APVMA). Although the majority of these products contain deet or picaridin as their active ingredients, there are a small number of registered repellents that contain botanical products. Some of these botanical-based products are widely available. There are also locally produced repellents sold direct to local stores, at community markets, and via online mail order. Few of these products have gone through the official registration process in Australia, either due to the ignorance of the manufacturers or due to the inhibitory financial costs of undertaking the required scientific studies of efficacy and health safety. These products typically contain extracts from one or more native Australian plants, as well as other aromatic essential oils, and are often marketed as "natural," "chemical free," "environmentally friendly," or "deet free."

In the absence of broad-scale mosquito control programs, many local authorities rely on the promotion of mosquito repellent use to minimize pest and public health risks. Although the use of deet- and picaridin-based repellents are routinely recommended, it is important that local authorities address the community's need for information on botanical-based repellents with the scientific evidence supporting deet- and picaridin-based repellents. Methodologies have been proposed for the testing of botanical repellents[13,14] that involve the use of internationally recognized mosquito species, such as *Aedes aegypti* (L.), that will provide a comparable measure of repellent efficacy across studies. Local authorities need access to accurate information that allows them to assess when, where, and how repellents containing extracts from Australian native plants sit within personal protection advice.

INDIGENOUS USE OF AUSTRALIAN PLANTS

The use of natural medicines by Australia's indigenous people has been well documented. Endemic plants species were used to treat many illnesses and ailments, from toothache to snake bite and from rheumatism to headache. Although there have been efforts to document the traditional use of native plants by Australian Aboriginal people,[15] there has been a substantial loss of knowledge regarding specific plants and their medicinal use since European settlement.[16]

Little is known of how mosquitoes or mosquito-borne diseases impacted Australian Aboriginal people prior to European settlement. There is also a paucity of documented evidence to suggest how these communities avoided mosquito bites. A range of plant species has been cited as having been used by Australian Aboriginal people as traditional medicines. Many of these were used for the treatment or prevention of insect bites. However, little evidence exists as to how these plants were used. There is no doubt, given the diversity of Australia's indigenous tribes, as well as plant species and geographic distribution, that there were differences in the types of plants used, and how they were used to protect against insect bites.

There is debate surrounding the impact of malaria parasites on the indigenous communities of Northern Australia before European settlement, but there is evidence that it was present and may have had some impact.[17] Although it has only been relatively recent that Australia's endemic mosquito-borne pathogens such as Ross River virus, Barmah Forest virus, and Murray Valley encephalitis virus have been documented, it is tantalizing to think that botanical medicines documented for use against rheumatism and fever[18] may have been used to treat symptoms we now know are associated with the human illness caused by these pathogens.[1]

Regardless of mosquito-borne diseases, given that many indigenous tribes spent time in and around Australia's coastal estuaries, there is no doubt that nuisance-biting mosquitoes, often present in large numbers, posed at least a pest problem.[19] The nuisance-biting impacts of Australia's mosquito fauna varies greatly across the country. Some of the most significant pest impacts are caused by mosquitoes associated with coastal estuarine environments such as salt marshes, mangroves, and coastal swamp forests. Key pest species include *Aedes vigilax* (Skuse) and *Aedes camptorhynchus* (Thomson).[1] These two species are often extraordinarily abundant when suitable environmental conditions occur. Given that Australian Aboriginal people spent much time in our coastal regions, it would be expected that they were exposed to substantial nuisance-biting by local mosquitoes.

Various types of substances have been used by indigenous cultures throughout the world to repel mosquitoes. These include smoke, plant extracts, oils, tars, and mud.[20] Some reports from Northern Australia have described the use of large circular pits covered by tea-tree bark, leaves, and sand to create a physical barrier against mosquitoes.[21] Some of the earliest records of Australian Aboriginals using plants for medicinal use came from Captain James Cook's journals of 1777 on the use of *Melaleuca* spp., and it was Cook who first used the term "tea tree" as his sailors brewed a tea from their aromatic leaves.[22] It had also been observed that young leaves of *Melaleuca* spp. were chewed to treat head colds by the Australian Aboriginal people.[18] This is interesting to note that many *Melaleuca* spp. dominate coastal swamp forests where mosquitoes can be abundant (Figure 10.1).

There are many examples of Australian native plants being used for medicinal purposes. However, there are very few examples of botanical products being used against biting insects.

Figure 10.1 The essential oils from tea tree and paperbark trees are often purported to be a safer and natural alternative to synthetic repellents. These plants, particularly those associated with coastal swamp forests in eastern Australia, are closely associated with productive habitats for pest mosquitoes. (Courtesy of Cameron Webb, Medical Entomology)

A summary of Australian native plant species purported to have some benefit in reducing the risk of mosquito bite, treating mosquito bite, or alleviating the symptoms of mosquito-borne disease is provided in Table 10.1. This list has been compiled from reports of plant use by both Australian Aboriginal people as well as early European settlers.

For many of these plant species, the exact use of the plant is not mentioned. For those plant species listed as an insect repellent, the leaves and/or bark of the plant may, most likely, have been burnt. Burning aromatic plants is a commonly described strategy to prevent mosquito bites in traditional cultures; there are even examples of the burning of plants to warn off the evil spirits responsible for illness. Without an understanding of the role of mosquitoes, perhaps this strategy assisted in reducing the mosquitoes spreading the disease-causing pathogens and has reinforced the practice.

Previous publications have suggested that the smoke produced from burning *Callitris intratropica* (Bowman and Harris) (Blue Cypress Pine) was used as a form of protection against biting mosquitoes.[23] Studies in Africa had shown that the smoldering leaves of an unspecified *Eucalyptus* tree repelled 72.2% of mosquitoes in human landing collections compared to untreated controls.

Table 10.1 List of Australian Native Plant Species Used by Australian Aboriginal People and Early European Settlers to Reduce Mosquito Bites or Treat the Symptoms Associated with Insect Bites or Illness

Scientific Name	Common Name	Reported Use
Acacia holosericea	Soapbush wattle	Treatment of insect bites
Alocasia macrorrhizos	Cunjevoi	Treatment of insect bites
Alstonia scholaris	Dita bark	Treatment of malaria
Banksia dentata	Tropical banksia	Insecticide
Callitris intratropica	Blue cypress pine	Insect repellent
Calytrix exstipulata	Turkey bush	Insect repellent
Carpobrotus glaucescens	Pigface	Treatment of insect bites
Eucalyptus globulus	Tasmanian blue gum	Insect repellent
Eremophila duttonii	Unknown	Insecticide
Kunzea ambigua	Tick bush	Insect repellent
Leptospermum liversidgei	Lemon-scented tea tree	Insect repellent
Lomatia silaifolia	Crinkle bush	Insecticide
Melaleuca alsophila	Saltwater paperbark	Insect repellent
Melaleuca cajuputi	Paperbark swamp tea tree	Insect repellent
Melaleuca quinquenervia	Broad-leaved paperbark	Insect repellent
Melaleuca styphelioides	Prickly paperbark	Insect repellent
Mentha australis	River mint	Insect repellent
Mentha diemenica	Slender mint	Insect repellent
Mentha satureiodes	Native pennyroyal	Insect repellent and insecticide
Ochrosia elliptia	Bloodhorn	Treatment of malaria
Ocimum acidula	Emu apple	Treatment of malaria
Ocimum sanctum	Sacred basil	Insect repellent
Petalostigma pubescens	Quinine berry	Treatment of malaria
Prostanthera cineolifera	Mint bush	Insect repellent
Pteridium esculentum	Common bracken	Treatment of insect bites
Pterocaulon serrulatum	Apple bush	Insect repellent
Triodia pungens	Soft spinifex	Insecticide
Santalum lanceolatum	Queensland sandalwood	Insect repellent
Spilanthes paniculata	Daisy cress	Insecticide

Sources: Lassak, E.V. and McCarthy, T., *Australian Medicinal Plants*, Reed New Holland, Sydney, Australia, 2001; Cribb, A.B. and Cribb, J.W., *Wild Medicine in Australia*, William Collins Pty, Sydney, Australia, 1981; Smith, N.M., *J. Adelaide Bot. Gard.* 14, 1–65, 1991.

This represented greater protection than a pyrethrin-based mosquito coil at 64.1% but less than a 19% deet-based topical repellent at 86.4%.[24]

Early European pioneers used *Mentha satureioides* R. Br. (native pennyroyal or creeping mint) to repel biting insects and *Mentha diemenica* Sprengel (slender mint) was spread on the floor to keep away fleas and other insects.[16] These species are closely related to the "pennyroyals" found in Europe and North America. The oil from these plants contains pulegone, a known insecticide. Pulegone is also found in *Nepeta cataria* L. (catnip) and *Mentha piperita* L. (peppermint), which have both been tested and found to have some mosquito repellent properties against Australian mosquitoes.[25] The small herb *Lomatia silaifolia* (Smith) R. Br. (crinkle bush) had been used to assist in preventing mosquitoes and nuisance flies indoors. Studies have shown that the flowers and nectar produce significant amounts of hydrogen cyanide.[18]

Some Australian native plants had been associated with the treatment and/or prevention of mosquito-borne disease. There have been suggestions that the bark of tropical rainforest tree *Alstonia scholaris* (L.) R. Br. (dita bark) has been used as an antimalarial, but clinical trials have shown that alkaloids in the bark of this, and the closely related species *Alstonia constricta* F. Muell. (bitterbark), have little antimalarial activity.[16] *Eucalyptus globulus* Labill (Tasmanian blue gum) had sometimes been referred to as "fever prevention tree" with a belief that the use of the oil prevented malaria. Similarly, many anecdotal reports of *Melaleuca quinquenervia* (Cav.) S. T. Blake (broad-leaved paperbark) may prevent disease by repelling mosquitoes.[16] Unfortunately, there is no current evidence that Australian native plants provide a suitable treatment for malaria.[26]

In addition to being used to repel mosquitoes, Australian native plants have been traditionally used to treat insect bites.[27,28] The two best-known examples of this are *Carpobrotus glaucescens* (Haw.) Schwantes (pigface) and *Pteridium esculentum* (J. G. Forster) Cockayne (common bracken). The young shoots of *Pteridium esculentum* are typically crushed and applied to the bite site, whereas the juice from the thick leaves of *Carpobrotus glaucescens* can be applied to bites. In addition, the sap of *Blechnum indicum* N. L. Burman (swamp water fern) has been reported as being useful for the treatment of insect bites. In recent years, essential oils derived from *Melaleuca* spp., marketed as "tea-tree oil" have been promoted widely as a "cure all" for a range of ailments, including insect bites.

In summary, although there has been a considerable loss of knowledge about the use of native plants by Australian Aboriginal people, there are indications that botanical repellents, either burning or as topical applications of crushed material, were used to prevent mosquito bites. The plant species listed in this section represent a small sample of plant species actually used by tribes throughout the region.

AUSTRALIAN NATIVE PLANTS TESTED FOR REPELLENCY

Essential oils derived from a wide range of native plants are commercially available and used for a wide range of purposes in Australia. Some are informally promoted in the local community as alternatives to synthetic repellents and are often perceived to be a safer alternative to synthetic products. Accurate information is required on the potential of these products to provide protection from biting mosquitoes so that they can be incorporated into the personal protection advice provided by local authorities. It is particularly important to avoid providing a false sense of protection to individuals and inadvertently increasing the risk of disease transmission.

Numerous studies have been conducted that have investigated the repellent activities of essential oils. These investigations have often been prompted by the use of endemic aromatic plant species by many cultures to protect against mosquitoes. The most significant factor influencing their effectiveness is the fact that they generally have low boiling points.[9] As they are highly volatile, the duration of protection against biting mosquitoes has generally been found to be poor compared to deet and

picaridin. However, some essential oils do demonstrate repellent activity and the problem of limited duration of activity may be addressed through improvements to the formulation process.

Prior to the availability of deet in the 1950s, the most common topical repellent used was citronella oil. It was first reported as being a suitable repellent in the early 1900s and subsequent research focused on the development of botanical-based repellents, particularly, citronella and pyrethrum oil-based products.[29] Much of the repellent research at this time concentrated on the use of botanical products and the development of techniques to prolong their effectiveness by mixing them with nonvolatile oils or greases.[30]

In the 1940s, Australian scientists tested extracts from Australian plants, alongside more than 125 substances that may act as repellents, against a range of mosquito species including *Anopheles farauti* Laveran s.l. *Aedes aegypti,* and *Aedes vigilax*.[30] A combination of laboratory and field testing was undertaken in a collaborative program between the Council for Scientific and Industrial Research and Australian Army Medical Corps. The most effective repellents were dimethyl phthalate; 2-ethylhexane-1,3-diol; Staway (a commercial repellent available at the time containing butyl carbitol acetate, carbitol, corn oil, and ethyl alcohol); and four native plant-derived substances, *Lagarostrobos franklinii* Hook. f. (quinn) [previously *Dacrydium franklinii* (Huon Pine)], *Melaleuca bracteata* F. Muell. (river tea tree), *Zieria smithii* Andrews (Sandfly Zieria), and *Backhousia myrtifolia* J. D. Hooker & Harvey (cinnamon myrtle).

Testing ranged from cage to tent to field situations and included a range of mosquito species. Interestingly, of the 18 Australian native plant-derived oils, most provided less than 20-minute protection against biting mosquitoes (Table 10.2). However, for *Dacrydium franklinii*, *M. bracteata*, *Zieria Smithii,* and *Backhousia myrtifolia*, protection times of more than 60 minutes were recorded in cage tests against *Aedes aegypti*. By modern standards, this would be considered a reasonable level of protection for botanical extracts.

Media reports of the testing results echo many of the findings of more recent research projects "some highly repellent oils from native plants are skin irritants and so are unsuitable for use. Others are pungent or produce nausea which renders them undesirable" (Cairns Post, Friday May 7, 1948).

Essential oils from Australian native plants have been included in a number of more recent broader screening studies. In particular, plants belonging to the Myrtaceae family, such as *Eucalyptus* spp. and *Melaleuca* spp., have been the primary focus. Overall, the repellency and protection times demonstrated by oils in these tests have varied greatly with *Melaleuca* spp. In screening of over 40 essential oils, *Eucalyptus globulus* Labill. (Tasmanian blue gum), *Eucalyptus divas* (broad-leaved peppermint gum) Schauer (broad-leaved gum), *Corymbia citriodora* (Hook.) K. D. Hill & L. A. S. Johnson (lemon-scented gum), *Eucalyptus radiata* Sieber ex DC. (narrow-leaved gum), *Melaleuca leucadendron* L. (cajeput), and *Melaleuca quinquenervia* (Cav.) S. T. Blake (Niaouli) were tested against three species of mosquitoes (*Aedes aegypti*, *Anopheles stephensi* Liston, and *Culex quinquefasciatus* Say). Although *Eucalyptus globulus* was one of the poorest performers in the testing against *Aedes aegypti*, the other Australian native plants performed well with protection times between 150 and 480 minutes against *Aedes aegpyti* and 330 and 480 minutes against *Anopheles stephensi*. Interestingly, all 41 essential oils tested against *Culex quinquefasciatus* provided 480 minutes protection from biting. The result for *Eucalyptus globulus* is supported by another study that showed that the essential oil provided no protection from bites in laboratory tests with *Aedes aegypti*.

There have been two studies that have specifically screened extracts from Australian native plants for potential use as insect repellents. The first of these tested a range of commercially available essential oils from Australian native plants.[31] Essential oils from a total of 11 Australian native plants were used in laboratory-based tests against *Aedes aegypti* and *Culex quinquefasciatus*. The essential oils tested were from *Agonis fragrans* J. R. Wheeler et N. G. Marchant (Fragonia), *Corymbia citriodora*, *Eucalyptus polybractea* R. Baker (blue-leaved mallee), *Eucalyptus radiata* Sieber ex DC, (Narrow-leaved peppermint) *Eucalyptus staigeriana* F. Muell. ex Bailey (lemon-scented Ironbark) F. Muell. ex Bailey (lemon ironbark), *Leptospermum petersonii* F. M. Bailey

Table 10.2 Protection Time Provided by Australian Native Plant Extracts Tested in Cage, Tent, and Field Situations in the 1940s

Scientific Name	Common Name	Cage Test[b] (Minutes)	Tent Test[c] (Minutes)	Field Test[d] (Minutes)
Atherosperma moschatum	Blackheart sassafras	40–60		
Backhousia myrtifolia	Cinnamon myrtle	>60	>60	40–60
Callitris glauca (leaf)	White cypress	<20	<20	<20
Callitris glauca (wood)	White cypress	20–40	<20	20–40
Doryphora sassafras	Sassafras	>60	40–60	
Eremophila mitchelli	False sandalwood	<20		<20
Eucalyptus dives	Broad-leaved peppermint	<20		
Eucalyptus dumosa	White mallee	20–40		
Eucalyptus radiata[a]	Narrow-leaved peppermint	<20		<20
Eucalyptus polybractea	Blue mallee	<20		
Eucarya spicata	Sandalwood	<20		<20
Lagarostrobos franklinii	Huon pine	>60	>60	>60
Melaleuca alternifolia	Narrow-leaved paperbark	<20		<20
Melaleuca bracteata	River tea tree	>60		40–60
Melaleuca ericifolia	Swamp paperbark	<20		<20
Melaleuca leucadendron	Cajepu	<20	20–40	<20
Melaleuca linariifolia	Narrow-leaved paperbark	20–40		20–40
Melaleuca uncinata	Broombush	<20		<20
Zieria smithii	Sandfly zieria	>60	>60	40–60

Source: Watanabe, K. et al., *J. Agr. Food Chem.,* 41, 2164–2166, 1993.
[a] Reported as *Eucalyptus phellandra*.
[b] Cage test results using *Aedes aegypti.*
[c] Tent tests conducted using *Aedes notoscriptus* and *Aedes alboannulatus.*
[d] Field tests conducted in NSW against a range of mosquito species including *Aedes vigilax, Aedes vittiger,* and *Aedes alternans* at biting rates of 25–50 per minute.

(lemon-scented tea tree), *Melaleuca alternifolia* (Maiden & Betche) Cheel (narrow-leaved paperbark), *Melaleuca ericifolia* Smith (swamp paperbark), *Melaleuca quinquenervia*, *Prostanthera melissifolia* F. Muell. (balm mint bush), and *Santalum spicatum* R. Br. (Australian sandalwood). All candidate repellents were tested as a 5% formulation in a carrier oil, *Simmondsia chinensis* (Link) Schneid (jojoba clear oil) that had been shown not to have any repellent properties.

Mosquito densities in the tests were altered to calibrate landing rates between the two species [*Aedes aegypti* ($n = 20$) and *Culex quinquefasciatus* ($n = 60$)] and their inherent differences in avidity. Mean protection time (MPT) provided by the oils ranged from 0 to 135 minutes in individual replicate tests, but there were differences in the response of each mosquito species (Figure 10.2). MPT provided by the extracts was generally greater against *Culex quinquefasciatus*. For the oils tested against *Aedes aegypti*, there was no significant difference in the MPT provided by the oils. However, the longest MPT was provided by *Melaleuca alternifolia* and *Leptospermum petersonii* for up to 35

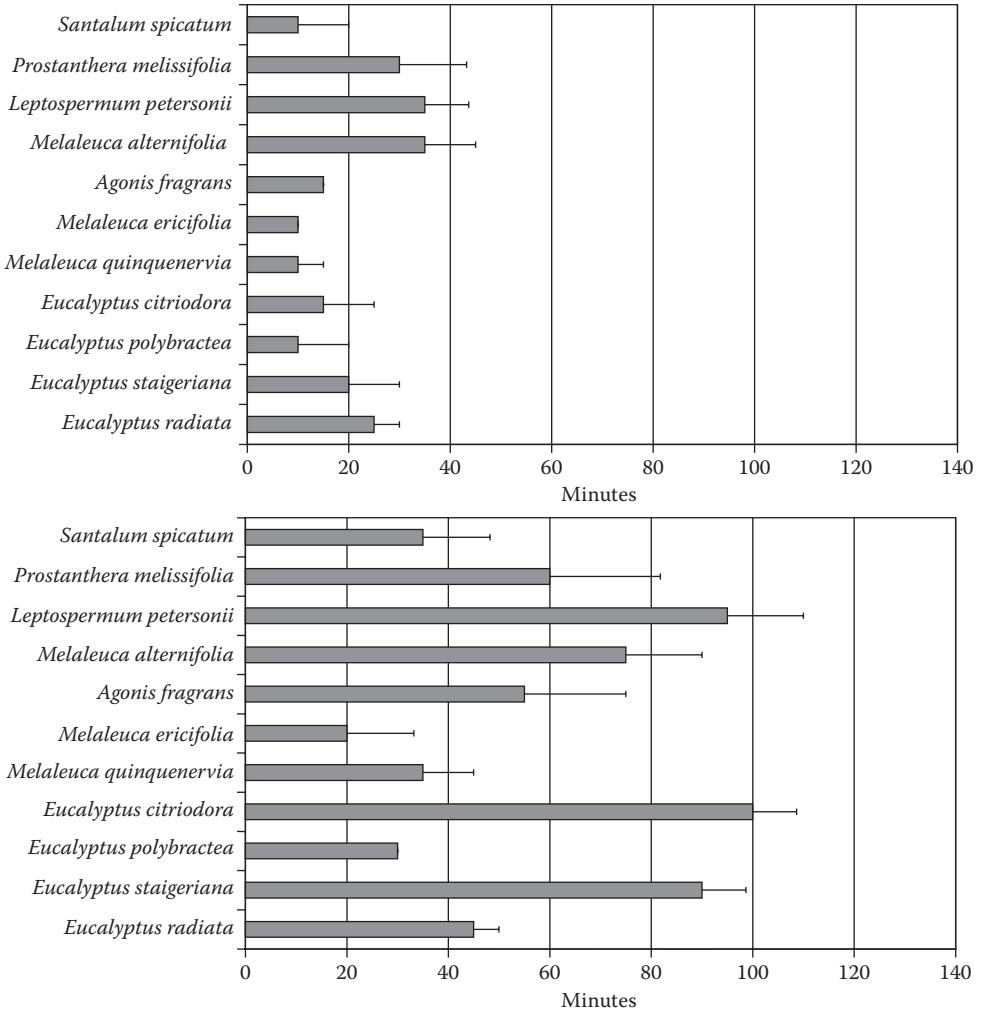

Figure 10.2 Mean protection time provided by a range of commercially available extracts from Australian native plants against (a) *Aedes aegypti* and (b) *Culex quinquefasciatus*. (From Maguranyi, S.K. et al., *J. Am. Mosq. Control Assoc.,* 25, 292–300, 2009.)

minutes. *Melaleuca ericifolia*, *Eucalyptus polybractea*, *Melaleuca quinquenervia,* and *Santalum spicatum* all provided the least protection against *Aedes aegypti* with an MPT of 10 minutes.

For the oils tested against *Culex quinquefasciatus*, the MPTs ranged from 20 to 100 minutes with the longest, 100 minutes, provided by *Corymbia citriodora*, which was significantly ($F_{10, 32} = 4.46$, $p = 0.002$) greater than *Prostanthera melissifolia*, *Agonis fragrans*, *Eucalyptus polybractea*, *Eucalyptus radiata*, *Melaleuca ericifolia*, *Melaleuca quinquenervia*, and *Santalum spicatum*.

In summary, the testing indicated that although not providing substantial protection against biting mosquitoes, some of the essential oils did provide some protection from biting mosquitoes. Further research is required to identify specific chemical components associated with native plant species that may hold greater potential than the essential oils themselves. The effectiveness of these repellents will be influenced further by formulations that regulate evaporation rates and subsequent protection times.[32]

It is possible that testing the potential repellency of these essential oils against an aggressive human biting species, such as *Aedes aegypti*, may underestimate the effectiveness of these products. For a more accurate indication of potential usefulness, field testing is required.

The second study[33] of Australian native plants involved initial screening of 10 plant species in laboratory tests against *Aedes aegypti*. The essential oils tested were from *Backhousia citriodora* F. Muell. (lemon-scented myrtle), *Syzygium anisatum* (Vickery) Craven & Biffen (Aniseed Myrtle), *Callitris columellaris* F. Muell. (Murray River cypress-pine), *Callitris glaucophylla* J. Thompson & L. Johnson (white cypress pine), *Eremophilla mitchelli* Benth. (false sandalwood), *Leptospermum liversidgei* R. Baker & HG Smith (lemon-scented tea tree), *Leptospermum petersonii* Bailey (lemon-scented tea tree), *Melaleuca linariifolia* Smith (flax-leaved paperbark), *Melaleuca ericifolia*, and *Melaleuca uncinata* R. Br. (broombush).

Each of the extracts was tested by exposing treated forearms to mosquitoes for 120 minutes with landing rates recorded every 30 minutes. MPTs were not recorded for this study, only repellency rates. The most notable finding of their study was that even on initial exposure of treated forearms to mosquitoes, no complete protection from landing mosquitoes was recorded for any of the candidate repellents.

Taking the results for the 30-minute exposure period, the most effective oils were *Melaleuca ericifolia* and *Callitris glaucophylla* that both provided over 80% repellency compared to an untreated control (Figure 10.3). The repellency rates dropped markedly after 30 minutes exposure with no essential oil providing over 60% repellency after 60 minutes and none providing over 50% after 120 minutes.

This study used a relatively high density of *Aedes aegypti* in testing cages of 50 mosquitoes. The mean landing rate on pretreatment and control exposures was between 24.8 and 44.8 mosquitoes per minute. For an avid biting mosquito such as *Aedes aegypti*, the relatively high biting pressures may have underestimated the potential protection times offered by the candidate repellents. When testing these candidate repellents against *Aedes aegypti* in cage trials, the results may underestimate the potential usefulness of these products under "real world" situations and field tests are required.

Taking the results from the laboratory tests, field testing of a 5% formulation of *Melaleuca ericifolia* (5% in either alcohol, emulsion, or gel) provided over 95% repellency (compared to untreated controls) up to 180 minutes against two common nuisance-biting species in the local area,

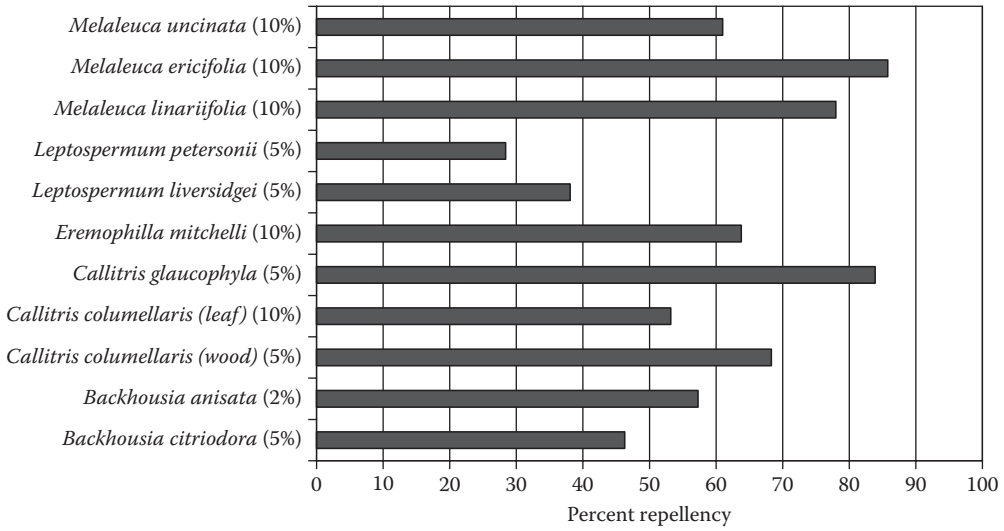

Figure 10.3 Percent repellency provided by essential oils from Australian native plants on forearms 30 minutes postapplication against *Aedes aegypti* in laboratory tests. (From Greive, K.A. et al., *Aust. J. Entomol.,* 49, 40–48, 2010.)

Aedes vigilax and *Verrallina carmenti* (Edwards), in 10-minute exposures of a treated lower leg of volunteers. These tests were conducted under reasonably high biting pressures in the field with pretreatment and control landing rates ranging from 3.68 to 5.60 mosquitoes per minute.[33]

Given that the botanical formulations performed as well as a 7% deet-based repellent for the first 3 hours of testing, it suggests that some comparable repellency is provided. However, it is important to note that no MPTs were provided in the study. Although no mosquito landings on individuals treated with the deet-based repellent occurred over the 3 hours of the study, some landings on the *Melaleuca ericifolia*-based repellent occurred after 60 minutes.

The implications of these two screening studies indicate that no essential oil from Australian native plants demonstrate a comparable level of effectiveness and duration of protection against biting mosquitoes to deet or picaridin. However, these tests indicate that there is some repellent activity displayed by these essential oils and perhaps in some circumstances may provide short-term protection.

Perhaps the greatest contribution by an Australian native plant has been *Corymbia citriodora.* It is a tall tree from temperate and tropical northeastern Australia. This tree species has previously been known as *Eucalyptus citriodora* but following a taxonomic revision, it has been reclassified into the genus *Corymbia*.

This plant occupies an interesting position among botanical repellents. Although the essential oil from this plant does not demonstrate substantial repellent activity, the by-product of the hydrodistillation process has been shown to be a very effective repellent. The active component is *p*-menthane-3,8-diol (PMD) and was first identified in China through a screening process of plants to identify potential repellent properties in the 1960s and is known as quwenling.

Laboratory and field tests have demonstrated that PMD effectively repels a range of mosquito species, including *Aedes* spp., *Anopheles* spp., *Culex* spp., and *Psorophora* spp.,[34,35] and biting midges.[36,37] Most importantly, studies have shown that PMD offers comparable, and often exceeds, protection times of deet against biting mosquitoes.

Botanical repellents are rarely endorsed by health authorities. However, PMD became the first plant-derived repellent to be included in public health messages issued by the Centers for Disease Control and Prevention (CDC) in North America. The inclusion of PMD in the list of recommended repellents provided by CDC is testament to both the effectiveness and safety of this product.[37]

Although research into Australian native plants is generally prompted by reports of use by Australian Aboriginal people, sometimes it is Australian wildlife that prompts investigation. *Kunzea ambigua* (Smith) Druce (tick bush) is an Australian native shrub whose common name comes from anecdotal reports that claim animals seek refuge from biting insects by sleeping beneath the shrub.[38] This is not the only example of Australian native plants being used by local wildlife to repel insects. The small shrub, *Eremophila duttonii* F. Muell., was traditionally known to be used by kangaroos in inland Australia to repel insects.[28]

Studies into the essential oil of *K. ambigua* have shown that the composition varies significantly between plants.[39] Gas chromatography–mass spectrometry analysis (GC–MS) of the oils from three *K. ambigua* cultivars showed clear compositional differences, particularly content of 1,8-cineole. In laboratory tests against *Aedes aegypti*, MPTs provided by a 40% and 60% formulation of *K. ambigua* remained below 40 minutes. However, there was no significant difference in between the MPTs provided by *K. ambigua* and the same concentrations of *Cymbopogon nardus* L. (citronella). It should be noted that there was very high biting pressure within the trials with landing rates on control forearms up to 59 ± 15 per minute and, as a consequence, may have underestimated the potential usefulness of this botanical repellent. Given the relatively high concentration of 1,8-cineole identified in one of the *K. ambigua* cultivars in this study, it would be interesting to have tested the oils at lower mosquito concentrations with a view to identifying any influence on protection times provided by differences in oil composition. In this study, the concentrations of both *K. ambigua and Cymbopogon nardus* tested were much greater than what would normally be

included in commercial botanical formulations where concentrations are generally less than 10%. At concentrations of over 40%, not only were the candidate repellents tested at a substantially higher concentration but the strong smell of the oils was noted to be unpleasant.

The repellency of extracts from *Eucalyptus camaldulensis* Dehn. (river red gum) have been tested. This tree can grow over 40 m and has become synonymous with inland water ways of Australia, particularly the Murray–Darling basin. In some parts of the world, this species is grown as a plantation timber. Two compounds, eucamalol and 4-isopropylbenzyl alcohol, were isolated from the leaves of the plant and tested for repellency against *Aedes aegypti* using chicks in laboratory tests.[40] Although no substantial repellency was provided by 4-isopropylbenzyl alcohol, after 2 hours, repellency rates of 87% (dropping to 75% after 3 hours) were recorded for eucamalol compared to 55% for deet. It is difficult to determine the significance of the result given repellency was tested under high biting pressure (500 mosquitoes in a 21 × 21 × 30 cm cage) and no concentration of the deet formulation was provided.

COMMERCIAL BOTANICAL REPELLENTS IN AUSTRALIA

All topical repellents must be registered with the APVMA before being commercially available. This process ensures that products are efficacious but also safe for human use. There are over 90 registered repellent formulations in Australia. The most common products are topical formulations containing deet and picaridin. These two active ingredients make up approximately 80% of commercial repellents available. The repellent activities of these two products have been shown to be effective against a range of nuisance-biting mosquito species.[11] Their effectiveness against Australian mosquitoes has also been demonstrated through extensive laboratory- and field-based assessments.[41,42] These two products are routinely promoted through public health messages issued by local authorities in response to increased mosquito risk during the summer months.

It is important for health authorities to provide clear and accurate information on the most suitable repellents. With this in mind, authorities must address the locally available repellents and respond with appropriate information in health warnings. There are currently 19 products registered with the APVMA in Australia that have botanical extracts as their active ingredients (Table 10.3). The majority of these are topical repellents containing Australian native plant extracts. There are currently only four formulations of topical repellents containing PMD registered for use. In Australia, PMD is listed as "Oil of Lemon Eucalyptus being acid modified extract of lemon eucalyptus (*Corymbia citriodora*)." As well as the topical repellents, there are also mosquito repellent wristbands containing peppermint or citronella and sandal wood sticks.

Despite completing the registration process, laboratory studies have demonstrated that "wrist band" formulations of repellents do not provide adequate protection against biting mosquitoes. In laboratory tests using bands containing a peppermint oil active ingredient against *Aedes aegypti*, some limited repellency was demonstrated but no protection from bites. The bands provided significantly greater repellency, based on mean landing rates, on the lower forearm where the band was worn compared to the upper forearm. The study demonstrated that the peppermint oil–impregnated wrist bands provided significantly less protection than a topical 7% deet-based repellent.

The "sandalwood stick" composed of compressed powered *Santalum spicatum* impregnated with citronellol, geraniol, and isopulegol to a total essential oil content of 5%. Field studies in North Queensland[43] against a range of nuisance-biting mosquito species including *Aedes vigilax*, *Aedes notoscriptus*, *Verrallina funerea* (Theobald), and *Verrallina lineata* (Taylor) found that while the burning of the sticks did not provide complete protection from biting mosquitoes, repellency rates compared to untreated controls of between 60.4% and 86.8% were recorded indicating some assistance in reducing mosquito bites was provided.

Table 10.3 List of Registered Mosquito Repellents That Contain Plant-Derived Active Ingredients

Component and Concentration	Name
Citronella oil (0.75 g/Band)	Bug Off Insect Repelling Wrist Band
Citronella oil (0.75 g/Band)	Mosquito-Band Anti-Insect Band
Citronella oil (0.865 g/L)	Avon Skin-So-Soft Bug Guard Mosquito Repellent Moisturising Spray
Citronella oil (15 g/Kg) + *Eucalyptus* oil (15 g/Kg)	New Mountain Sandalwood Sandalwood Mosquito Sticks
Citronella oil (25 g/L)	Roonka Australia Personal Insect Repellent
Citronella oil (25 g/L)	Vamoose Personal Insect Repellent
Citronella oil (30 g/L) + *Melaleuca* oil (20 g/L)	Tallebudgera Herbals Herbal Blend Insect Repellent
Lemon Eucalyptus extract (300 g/Kg)	Mosi-Guard Personal Insect Repellent Lotion
Lemon Eucalyptus extract (320 g/Kg)	Mosi-Guard Personal Insect Repellent Roll-On
Lemon Eucalyptus extract (338 g/Kg)	Mosi-Guard Personal Insect Repellent Aerosol Spray
Lemon Eucalyptus extract (400 g/Kg)	Mosi-Guard Personal Insect Repellent Spray
Melaleuca oil (18.9 g/L) + *Leptospermum* oil (9.45 g/L) + Citronellal (28.35 g/L)	Thursday Plantation Australia's Original Walkabout Insect Repellent
Melaleuca oil (50 mg/G)	Ego Naturals Moov Insect Repellent Gel Natural Active Ingredient
Melaleuca oil (50 mg/G)	Ego Naturals Moov Natural Insect Repellent Spray Natural Active
Melaleuca oil (50 mg/G)	Ego Naturals Moov Insect Repellent Roll On Natural Active
Peppermint oil (100 g/Kg)	Gone Insect Repellent Band
Citronella oil (25 g/L)	YaMate Personal Insect Repellent
Citronella (30 g/L) + *Melaleuca alternifolia* oil (20 g/L)	Natralia Nourish Naturals Insect Repellent

Source: Compiled from information provided on the website of APVMA, http://www.apvma.gov.au/, accessed November 10, 2012.

Although a number of studies have investigated the effectiveness of registered repellents in Australia, few have tested those containing botanical active ingredients. In the absence of comparisons between the commonly registered products in Australia, it is difficult for local health authorities to provide adequate advice on repellent use.

Laboratory tests were conducted using a range of registered topical repellents containing botanical extracts, deet and picaridin, to determine the relative protection provided by these different products. All repellency testing was undertaken under laboratory conditions at an average temperature of 26.5°C ± 2°C and approximately 70% relative humidity. Human volunteers applied 1 mL of the repellent to one forearm, leaving their second forearm as an untreated control. Control and treated forearms were then exposed to a cage (40 × 40 × 40 cm) containing 20, four- to seven-day-old adult female *Aedes aegypti* for either 3 minutes (treatment) or 1 minute (control). The number of landing mosquitoes on treated and untreated forearms was recorded and the procedure was repeated five times with 15-minute intervals between each exposure. Forearms were exposed to the cage of mosquitoes until three "bites" were received. "Bites" were classified as a mosquito probing before commencement of blood feeding. At this point, the experiment was terminated and the time was recorded. Each repellent was tested on five volunteers using a randomized design, with only one treatment combination tested per day.

The results of the testing are presented in Figure 10.4 Unsurprisingly, repellents containing 17%–20% deet provided the longest duration of protection, between 372 and 406 minutes. There was little difference in the MPTs provided by repellents containing between 5.2%–10% deet, 9.2%–10% picaridin, and 30% PMD. This result confirms findings from both field and laboratory tests elsewhere that PMD, although at a higher concentration than deet or picaridin, can provide comparable levels of protection against biting mosquitoes.

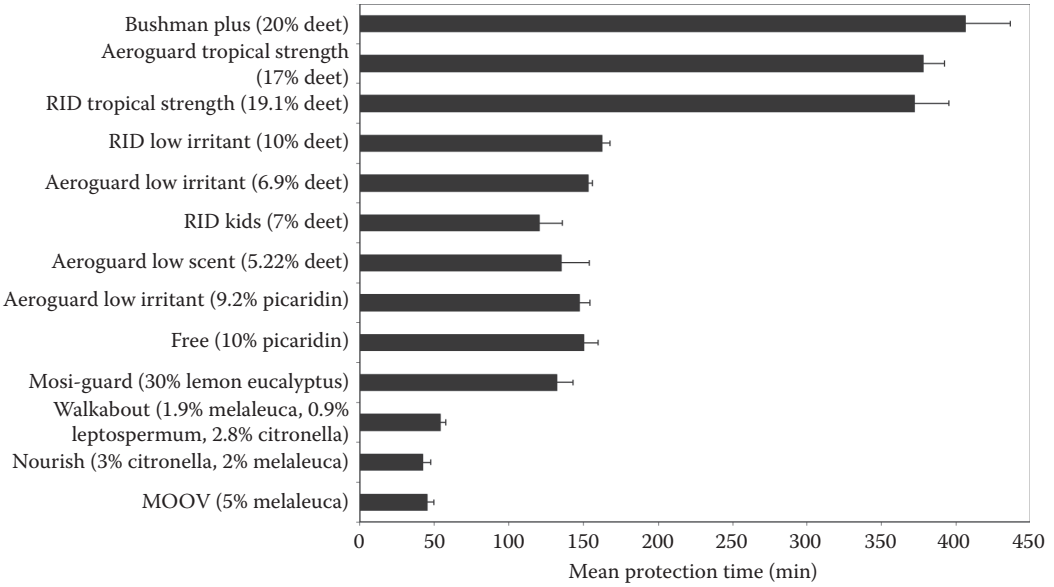

Figure 10.4 Mean protection time provided by a selection of topical mosquito repellents registered for use in Australia by the Australian Pesticides and Veterinary Medicines Authority compared to deet- and picaridin-based repellents tested against *Aedes aegypti* in laboratory tests.

The three repellents tested that contained Australian native plant extracts all provided between 42 and 52 minutes protection. This level of protection was substantially less than the low concentration formulations of deet and picaridin and the 30% PMD repellent. However, the tests demonstrate that there is some repellent activity provided by the botanical repellents.

Extension of the research into field trials is necessary to test the products under more realistic pest densities and biting rates. As highlighted in previous studies, testing repellents against *Aedes aegypti*, while considered a recommended methodology for botanical repellents,[14] may underestimate the repellent activity provided by these products.

IMPLICATIONS FOR PUBLIC HEALTH MESSAGES

Recommendations on personal protection strategies are always included in messages from local authorities around awareness of nuisance-biting mosquitoes and mosquito-borne disease risk. These messages, however, often do not reflect the wide range of formulations and active ingredients available to the consumer, not to mention the issues surrounding unregistered products. Authorities need to respond to the variety of products available as well as those that have been tested for efficacy.

Repellents come in many different formulations including pump sprays, aerosols, lotions, roll on, wipes, area repellents (coils and sticks), laundry fabric treatments, and more recently in combination with cosmetic products. The demands from consumers for products that are more pleasant to use have led to an increase in the range of deet-based repellents containing less than 10% deet. These products are often marketed as "low scent" or "low irritant" formulations. However, there is also a growing resistance to the use of these chemical repellents by some sectors of the community due to perceived adverse health effects.[8] Despite repeated assessments of deet, as well as picaridin, showing that these topical repellents pose no significant health risk,[7,44] many in the community have a desire to use natural products.

Although essential oils and botanical-based repellents are often perceived as a safer alternative to deet- or picaridin-based repellents, it is important that the community is educated on the issues surrounding the use of these products. Dermatological reactions to essential oils have been reported, including products associated with purported insect repellency such as sandal wood.[45] However, some of the most serious adverse health impacts have resulted from the use of *Melaleuca* and *Eucalyptus* oils.[46,47]

Essential oils derived from *Melaleuca* spp. and, occasionally, *Leptospurnum* spp. are marketed as "tea-tree oils." They have become popular throughout the world as a "cure all" for a wide range of ailments including the prevention and treatment of insect bites. Tea-tree oil may contain extracts from a number of *Melaleuca* spp. but, typically, it is derived from *Melaleuca alternifolia*.[22] This species has been identified as having antibacterial activity[48] that has contributed to its popularity, but has also been identified as posing potential health risks.

There have been a number of case reports of adverse health impacts resulting from the topical use of *Melaleuca* oil or its ingestion. This oil contains approximately 100 compounds and, although a number of cases of dermatological reaction have been reported, the actual compound responsible has varied between patients in patch testing.[22] A review[47] of the toxicity and allergy studies of dermal exposure to *Melaleuca alternifolia* highlighted the variability of individuals' response to the oil but does note that this product does have potential to adversely impact some users.

Fact sheets and media releases released by the six state and two territory health departments in Australia, along with local governments, often do not reflect the range of repellents currently registered or shown to be effective. All health authorities mentioned recommend a deet-based repellent and of those that suggest an appropriate concentration, a 20% formulation is recommended. Although this advice is supported by studies that show a 20% deet- or picaridin-based repellents will provide substantial protection against biting mosquitoes, the majority of products available generally contain less than that concentration. The prevalence of deet- and picaridin-based repellents containing approximately 10% active ingredient has the potential to leave consumers confused.

Health authorities in Australia generally make no mention of botanical-based repellents. In the review of studies on the efficacy of botanical-based repellents, it is clear that they provide substantially less protection than deet- or picaridin-based products. However, there is still some repellent activity provided and, in some situations, may provide suitable short-term protection against mosquito bites. As these products have completed the registration process with APVMA, it may also cause confusion among consumers as to why these products have been considered suitable for use but not recommended by health authorities.

In the case of botanical-based repellents, perhaps an option for health authorities is to move to a model of recommendations based on reapplication times. This system generally works well for public health messages around sunscreen use in Australia[49] and perhaps by stressing a reapplication time for botanical-based products compared to synthetic-based products, consumers may be better informed. For example, health messages may include statements such as "botanical-based repellents must be reapplied twice as often as 10% deet or picaridin based repellent and four times as often as a 20% deet or picaridin based repellent." The messages around repellent use need to be refined as there is likely to be some confusion regarding what labeling on commercial products means with regard to protection from bites. Most importantly, it should be stressed that the percentage of repellent relates to protection times (perhaps better explained as reapplication times) not the quantity of mosquitoes that are repelled. This would further enhance the messages around the limited protection provided by botanical-based products.

Regardless of changes in the relative risks associated with exposure to mosquitoes in the future, either through the introduction of exotic vectors and/or pathogens or shifts in the abundance and/or distribution of local mosquitoes, the provision of informed advice on the use of topical insect repellent will remain critical. Local health authorities must have an adaptive approach to public health messages so that appropriate advice is provided on new commercial mosquito repellent

formulations. Formulations that combine repellents with sunscreen, or other cosmetics, must be assessed[50] as do "spatial" repellents such as wrist bands[51] as there is often great interest from the public in these repellents. If advice on topical repellents is to have any impact on rates of mosquito-borne disease, authorities must consider local risk factors and the current availability of mosquito repellents.[52]

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CHAPTER 11

Topical Repellent Active Ingredients in Common Use

Daniel Strickman

CONTENTS

INTRODUCTION

This chapter describes a wide variety of chemicals that disrupt arthropod blood feeding. The exact mechanism of action of these compounds is still uncertain, but they all have the net effect of stopping bites. Many chemicals discovered so far give the hope that there is a compound that is far more effective than current active ingredients. At this time, five compounds are in common use, though there are others with a smaller market share (e.g., 2-undecanone[1]). The five compounds include one that was derived botanically (*para*-menthane-3,8-diol [PMD]), two developed through molecular modeling (picaridin and IR3535®*), one discovered by screening many possible compounds (deet), and one derived from a similar chemical structure (*N,N*-diethyl phenylacetamide [DEPA]). This chapter reviews the basic properties and effectiveness of these five compounds.

DEET

Deet is *N,N*-diethyl-3-methylbenzamide (*N,N*-diethyl-m-toluamide).[2] It was the result of a series of tests with similar compounds beginning about 1942, with a patent applied for by Samuel I. Gertler in 1944 (it was granted in 1946). Those compounds irritated the skin, and it was not until 1952 that the U.S. Department of Agriculture laboratories in Beltsville, Maryland, and Orlando, Florida, identified deet as an active ingredient that provided much longer protection than dimethyl

* A registered trademark of Merck, Whitehouse Station, New Jersey.

phthalate, indalone, and ethyl hexanediol, which were the most effective repellents at the time.[3] Deet was on the market in 1956 and came to dominate repellents by 1970. The compound had the advantages of longer duration and broader spectrum of effectiveness than the previous standard active ingredients.[4] Because of its long history of use and because it has come to be the gold standard against which other repellents are compared, deet has been tested against many different kinds of arthropods. As a result of its rich history of testing, deet's weaknesses and strengths are documented. The published record for other common active ingredients is not as extensive.

The safety of deet has been reviewed extensively,[5–10] as would be expected for a compound used in literally billions of applications. The generally good safety record for deet was confirmed by a reregistration document prepared by the U.S. Environmental Protection Agency (EPA). The reregistration confirmed the satisfactory safety of deet, even when applied as the 98% technical material.[11] The lack of toxicity is significant because 10%–15% of typical formulations are absorbed through the skin, metabolized, and excreted.[12] Formulation can have a big influence on absorption. For example, alcohol formulations were more readily absorbed,[13] whereas special formulations of deet in liposomes resulted in minimal absorption.[14] Adverse effects from deet may be the result of neurotoxicity in response to high doses taken orally by suicide victims.[15] Significant inhibition of acetylcholinesterase[16] attracted a great deal of public attention and also several questions from the scientific community about the appropriateness of the dose in the study by Corbel et al.[47] Skin irritation has been observed on sensitive areas such as the inside of the elbow, especially when the area was occluded by an impermeable object.[12,17–20] Toxicological interaction between deet and the acetylcholinesterase-blocking drug pyridostigmine was suggested as a cause of Gulf War syndrome,[21] but the studies used very high doses of deet to see this effect.[10] No interaction has been seen between deet and permethrin.[6] Adverse effects on a fetus were also absent, despite finding residues in fetal blood following application to the pregnant mother.[22]

The efficacy of deet is extremely broad.[2,23] The chemical prevents biting by most mosquitoes (Culicidae), black flies (Simuliidae), sand flies (Psychodidae: Phlebotominae), biting midges (Ceratopogonidae), chiggers (Trombiculidae), and hard ticks (Ixodidae). It also prevents attachment by land leeches (Annelida: Hirudinea) and penetration of skin by schistosomal cercariae (Trematoda: Schistosomatidae). It also discourages biting by stable flies (*Stomoxys calcitrans*) and tabanids. Despite its amazing range of effectiveness, deet fails to repel or only weakly repels some important biting arthropods: soft ticks (Argasidae), kissing bugs (Reduviidae: Triatominae), and lice (Phthiraptera) appear to be tolerant to the repellent. *Anopheles* mosquitoes are generally repelled for a shorter time than either *Culex* or *Aedes.* Deet-based repellents last a distinctly shorter time against the common malaria vector of Central America *Anopheles albimanus.* This species is susceptible in that it does not bite for 1 or 2 hours after application, but repellency apparently depends on the presence of a higher concentration than for other kinds of mosquitoes. The susceptibilities of bed bugs, stable flies, tabanids, and tsetse (*Glossina* spp.) are similar to *Anopheles albimanus* in that they usually do not bite when deet is present at a high enough concentration, the practical effect being that products do not provide very long-lasting protection.

DEPA

DEPA was developed in India,[24,25] motivated by the relatively high cost of deet synthesis in the country. When first developed, DEPA cost 86% less than deet to make in India. The compound had actually been evaluated in the 1940s by the U.S. Department of Agriculture laboratory in Orlando but not developed further at that time.[23]

The U.S. EPA has not evaluated DEPA for registration, presumably because no commercial entity has attempted to do so. The material has been registered in India and has been shown to be sufficiently nontoxic for application to human skin.[26] Although safe, DEPA is generally more toxic

than deet in standard toxicological tests. For example, it is over twice as toxic by the oral route in rats.[27] DEPA, similar to deet, causes irritation of mucous membranes when directly applied to them.[28] It is also mainly eliminated through the urinary route following rapid metabolism of the compound absorbed through the skin.[29]

The efficacy of DEPA appears to be almost identical to that of deet. Studies of mosquitoes (*Anopheles culicifacies* and *Culex quinquefasciatus*), sand flies (*Phlebotomus papatasi*), black flies (*Simulium himalayense*), tropical bed bugs (*Cimex hemipterus*), fleas (*Xenopsylla cheopis*), and leeches showed 2–8 hours of protection on skin. DEPA was also effective on clothing. Overall, DEPA is a useful active ingredient as a repellent.[26] Another very similar compound, *N*,*N*-diethyl benzamide, has also been developed into a product in India.[30]

IR3535

IR3535 is Merck and Company's code for ethyl butylacetylaminopropionate or EBAAP. It is synthetic but derived from β-alanine, a naturally occurring amino acid. IR3535 was developed by molecular modeling of other repellent compounds.[31] It was not introduced into the United States until 1999, although it was used in many products in Europe for 15 years before that. Although relatively few American products contain this active ingredient, it retains a significant market share probably because of its excellent application characteristics.

Unlike the other active ingredients that have an oily feel, IR3535 is more miscible with water and is largely undetectable on the skin after a product dries. There have been no adverse reactions from this active ingredient, and its toxicological profile describes an active ingredient that is practically nontoxic.[32] The rat oral lethal dose (LD_{50}) is reported to be more than 5000 mg/kg, dermal LD_{50} of 3000 mg/kg, showing no sign of skin sensitization. It caused no dermal irritation in rats, although it did irritate rabbit eyes.

Efficacy of IR3535 is controversial. On the one hand, there are abundant studies showing an even broader range of effectiveness than deet.[33] In fact, the number of published laboratory and field studies on IR3535's effectiveness rival those on deet. Overall, the protection times against mosquitoes were 10%–20% less than for equivalent concentrations of deet and highly dependent on formulation. Nonetheless, products with 20% IR3535 provided over 8 hours of protection in most tests. Similar to deet, IR3535 was less effective against *Anopheles* mosquitoes than against *Culex* and *Aedes.* Some authors have concluded that IR3535 is less effective than deet against mosquitoes,[34,35] whereas others have shown its equivalence to picaridin.[36] IR3535 also inhibits biting by stable flies, tabanids, black flies, and biting midges. There is convincing evidence that it is superior to deet for the prevention of *Ixodes* tick bites.[37,38] Unlike deet, IR3535 is an effective repellent against head lice (*Pediculus humanus capitis*), bees, and wasps.

PICARIDIN

Picaridin [2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester] was developed by Bayer AG, Leverkusen, Germany, through molecular modeling and very thoroughly tested before it was released on the European market in 2001. It did not appear in the United States until 2005 and is the first synthetic repellent to go through newer standards of toxicological testing of the U.S. EPA as a part of its initial commercial development. Picaridin has several other names. Its developmental reference code, KBR 3023, is still seen on some documentation. The World Health Organization uses the name "icaridin," and Bayrepel® was a registered trademark of Lanxess Corporation. Most recently, the compound has been called Saltidin® by the Saltigo Company, a member of the Lanxess group. Picaridin is now widely used, being marketed in over 50 countries.

The toxicological profile of picaridin is similar to that of IR3535, indicating that it is a very safe compound. High dosages in animals show no effect on the nervous system, but they have caused damage to liver and kidneys. There is one published record of an adverse effect associated with contact allergy.[39] A quantitative evaluation of margin of exposure based on exposure from actual products showed that picaridin had a wider margin of safety for acute toxic effects but a narrower margin for subchronic and chronic effects.[40] Picaridin is oily, not a plasticizer, with a less objectionable odor than deet.

The effectiveness of picaridin is excellent.[41] The compound prevents bites from the same spectrum of biting pests as deet. The duration of protection against *Anopheles* is less than that for other mosquitoes, and protection against ticks is markedly less than that against mosquitoes. In general, consumers can expect 2–5 hours of protection from mosquitoes when using 10% formulations and 6–10 hours of protection when using 20% formulations.[23] In general, picaridin is considered to give about the same level of protection as deet against biting arthropods. It often provides 10%–20% longer protection at the same concentration. Anecdotally, picaridin seems to keep blackflies and mosquitoes at a greater distance than deet.

PMD

PMD, or *p*-menthane-3,8-diol (CAS 42822-86-6), was originally rejected by the U.S. Department of Agriculture as a promising repellent active ingredient in 1955. It was later identified as the major component of a local Chinese repellent, quwenling.[42,43] Quwenling is the steam distillate from lemon eucalyptus, *Corymbia citriodora*; therefore, the active ingredient is sometimes listed as "oil of lemon eucalyptus," although the material is not technically an essential oil. True oil of lemon eucalyptus is a separate material (CAS 129828-26-6) and includes a mixture of terpenes. Quwenling is not pure PMD, as it also includes a mixture of terpenes that may contribute to repellency. PMD can be synthesized, cheaply produced from any of a number of related botanical compounds, or extracted from the plant. It is a solid at room temperature and has an aromatic odor, which is unusual for a repellent. Some preparations have a strong eucalyptus odor, perhaps intentionally.

Although most of the toxicological indications for PMD are extremely mild,[44] its concentrate has received the worst category as a poison (Category I: Danger) because the material is very damaging to the eyes. Diluted formulations seem to be safe, but American labels warn against getting them into the eyes. Botanical extracts may contain as much as 60% PMD, so the danger to the eyes may be an important issue for some products. To some extent, toxicological testing of PMD has not been as thorough as for the other compounds considered in this chapter because this active ingredient is considered natural. Nonetheless, PMD has achieved U.S. EPA registration based on its safety to the user and the environment.

Efficacy of PMD is similar to that of deet against mosquitoes.[35] It appears to be superior to deet against *Anopheles* mosquitoes and possibly against stable flies and biting midges. The greater effectiveness against *Anopheles* has stimulated trials for malaria control, which have been successful when the material was used in conjunction with treated bed nets.[45] A formulation of PMD and lemongrass oil appears to be particularly effective and very acceptable to users.[46]

CONCLUSION

For decades, deet was the clear choice for the most effective repellent active ingredient worldwide. Currently, there are at least four alternatives to deet, including two that claim a natural origin. Each material has its advantages and disadvantages (Table 11.1), but the use of any of them will provide considerable protection from a wide variety of pests. Possibly the most controversial active ingredient is IR3535. Its excellent safety and application characteristics are superior to the others, but its effectiveness appears to be highly dependent on formulation. The data are sparse, but IR3535

TABLE 11.1 Comparison of Major Topical Repellent Active Ingredients

Ingredient	Known Effectiveness Against	Advantages	Disadvantages
Deet	Chiggers, biting mites, mosquitoes, biting midges, black flies, sand flies, stable flies, horse flies/deer flies, tsetse flies, fleas	Cheap, long safety and evaluation record, very-broad-spectrum protection	Oily; distinct odor; melts plastics; irritates eyes; not as effective against ticks, kissing bugs, malaria mosquitoes
Picaridin	Ticks, chiggers, biting mites, mosquitoes, biting midges, stable flies, fleas	Very broad spectrum, does not melt plastics, low odor, not as oily, works at lower concentrations	More expensive; less experience with use; not as effective against ticks, some malaria mosquitoes, and biting midges
IR3535	Ticks, chiggers, biting mites, mosquitoes, biting midges, sand flies, horse flies/deer flies	Extremely safe, long evaluation record, low odor, not oily, does not melt plastics, broad spectrum	Sometimes fails at low concentrations
DEPA	Ticks, chiggers, bed bugs, biting mites, mosquitoes, biting midges, blackflies, sand flies, stable flies, horse flies/ deer flies, fleas	Cheap, broad spectrum	Oily, distinct odor, melts plastics
PMD	Ticks, mosquitoes, biting midges, flies	Good against malaria mosquitoes and ticks, botanical derivative	Only partially evaluated, some preparations have strong odor, irritates eyes

Source: Strickman, D. et al., *Prevention of Bug Bites, Stings, and Disease*, Oxford University Press, New York, 2009.

appears to be better against ticks than other repellents and might be a clear choice for protection from tick-borne pathogens. PMD might be the best choice against *Anopheles* vectors of malarial parasites. Deet, DEPA, and picaridin are all very good, general-purpose repellents that will probably remain useful for many years to come.

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CHAPTER 12

Spatial or Area Repellents

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CONTENTS

INTRODUCTION

Spatial or area repellents create a three-dimensional zone from which biting arthropods are repelled or within which they fail to bite. The potential advantages of spatial repellents include protection of several people with a single product, ease of use without the need to make applications to the skin, and a continuous level of protection. People have probably used smoke as a spatial repellent for tens of thousands of years and historical records have shown the use of particular plants since ancient times. The subject of this chapter includes the various systems for dispersing chemical spatial repellents. Physical systems can be as simple as fans or screened enclosures, or as complicated as radio-frequency generators, but these are not discussed here.

Development of spatial repellents has been a consistent goal for those interested in personal protection from flying insect bites. Spatial or area effects from repellents were reported by Christophers in 1945.[1] He especially noticed the actions caused by pyrethrins. Christophers also noted the distinction between "contact" and "vapor" repellents as described by McCulloch and Waterhouse.[2] The terminology associated with spatial repellency has evolved through time. Bernier et al.[3] discussed some of these changes and provided a brief early history of research efforts in the field of spatial repellency. Strickman[4] also reviewed spatial repellents. As an offshoot from their well-established program of topical repellent testing, a concerted search for spatial repellents was undertaken by the U.S. Department of Agriculture in 1948. "Spatial" was defined by Gouck et al.[5] as a compound

or agent that could produce repellency at a distance. Nolen et al.[6] later defined spatial repellents as volatile chemical compounds that possess the ability to inhibit the host-seeking behavior of insects in an environmentally defined three-dimensional space. Recently, Bernier et al.[3] have shifted away from the use of the term "spatial repellent" for human-produced masking chemicals in favor of the term "attraction-inhibitors." They believe this term to be a logical choice to describe the behavioral effect (inhibition) observed in bioassays.[7,8] Some researchers have used the term[9] "noncontact irritancy" to describe the behavior of mosquitoes moving away from aerially dispersed insecticide.[9–12]

Authors agree that spatially active chemicals prevent biting by the disruption of normal vector behavioral patterns that would otherwise occur sequentially over a considerable distance. Those patterns include detection of the host, orientation toward it, and the actual approach to the host as a potential blood-meal source.[12] As a result, the three-dimensional space around the potential host is made unsuitable for the vector well before the arthropod reaches the skin. All spatial repellent systems attempt to prevent blood-feeding arthropods from reaching their target hosts within a space of concern. This is in contrast to the topical repellents that are placed on the skin or clothing, requiring either contact by the arthropods or very close proximity to stop them from biting at the very last stages of their feeding processes. In effect, topical repellents are highly targeted, as active ingredients are applied exactly where they are needed: at the site of a potential bite. Therefore, in this chapter, for the purposes of clarity as suggested by Ogoma et al.,[13] "spatial repellency" is used as a general term to refer to the sum of mosquito behaviors (e.g., causing movement away from the chemical stimulus, interfering with host detection by attraction-inhibition,[3] deterrents,[14] and/or feeding response) induced by airborne chemicals that prevent mosquitoes from making physical contact with the host.

Regardless of the particulars, the general concept of spatial repellency is clear: to discourage a biting arthropod from entering a space occupied by a potential host. The intended practical result is to reduce encounters between hosts and vectors, hopefully lowering the risk of disease from pathogens transmitted by arthropods. A unique benefit of spatial repellency is that the safe zone can include areas both indoors and outdoors. The volume of space that is protected, or minimum protection range, will be dependent on the properties of the active ingredient, application platform, and environmental conditions (e.g., airflow, temperature, and humidity). An area repellent does not necessarily require a significant vapor phase to achieve a spatial repellent effect. For example, the use of a repellent that is normally applied topically, such as deet, on a treated net can form a barrier around a perimeter.[15]

KINDS OF SPATIAL REPELLENT PRODUCTS

The public's desire for a product that acts as a spatial repellent is reflected in the large number of products available. These products release the active ingredient into the area either passively, through unaltered volatilization and diffusion, or actively by applying heat, mechanical aerosolization, or volatilization enhanced by airflow.

Active-Emission Devices

Mosquito coils are the most popular spatial repellent product. They use any of a variety of active ingredients impregnated in a slow-burning medium. Part of their popularity is based on the low cost of manufacture and the low cost of use. A single coil might last for up to 8 hours and cost less than US$1.00. In an elegant bit of physics, the active ingredient is not burned, but released behind the smoldering tip of coil, as the temperature for volatilization is achieved. Therefore, the volatility and heat stability of a particular active ingredient is not critical, as it will volatilize at the time when the right temperature reaches it.

The effectiveness of coils has been evaluated in several ways that measure either knockdown or suppression of biting. Often, knockdown effectiveness is expressed as the time required for killing or incapacitating the target arthropods. Suppression of biting is usually expressed as the percentage of bites prevented during a period compared to an untreated control. That measurement is a great contrast to standard tests of topical repellents. Topical repellents are tested with the assumption that protection will be complete, but that the duration of protection will vary from product to product. The percentage suppression of biting by coils and other spatial repellents over a period reflects the assumption that protection will be consistent as long as the coil is burning, but that protection will not be complete. Knockdown times are surprisingly long for pyrethrum-based (0.35%) coils, with reports for *Aedes aegypti* varying between 16.5[16] and 20.3 minutes.[17] The same kind of coil prevented 42% of the bites from *Culex quinquefasciatus*.[3] Coils with 0.5% pyrethrum prevented the entry of 58% *Anopheles gambiae*, 56% *Mansonia uniformis*, and 65% *C. quinquefasciatus* into verandah traps.[18] Commercial coils containing 0.072% allethrin, 0.05% *S*-bioallethrin, or 0.16% pyrethrins prevented 85% or more of the bites from *Anopheles stephensi* on a guinea pig in the laboratory.[19] Coils containing either allethrin or D-*trans*-allethrin at concentrations varying between 0.12% and 0.28% prevented 71%–75% of bites from *C. quinquefasciatus* during 8 hours.[20] A coil containing 26% permethrin and placed immediately upwind of collectors prevented 62% of the bites from a mixed population of *Aedes punctor* and *Aedes communis*.[21]

Ogoma et al.[13] conducted a systematic review of the literature on mosquito coils and passive emanators. They reported that despite differences in evaluation methodologies, coils and emanators clearly reduce human–mosquito contact. They induce mortality, deterrence, repellency, and reduce feeding of mosquitoes on humans. Although organochlorine insecticides (lindane and dichlorodiphenyltrichloroethane [DDT]) were used at one time,[19] various pyrethroid chemicals have been common active ingredients in coils since at least the 1930s.[20] Among the most common ingredients currently used are pyrethrum, prallethrin, transfluthrin, allethrin, and esbiothrin. The pyrethroids have a stronger "expellent" effect than other commercial insecticides, tending to act as a true area repellent, rather than a fumigant insecticide.[22] Natural pyrethrum varies in composition and has been considered superior to[16] or inferior to[19] some synthetic pyrethroids in mosquito coils. Smokeless generation of repellent vapors by candle, butane catalyst, or electrical plug-in devices is generally as effective as coils. Such devices have provided up to 98% protection from the bites of the sand fly, *Phlebotomus papatasi* (butane-powered),[23] 97% protection from *Aedes caspius* (butane-powered),[23] 59%–78% protection of chickens in the laboratory from *Anopheles stephensi* (electrically heated),[24] and 40%–79% protection of chickens from *C. quinquefasciatus* (heated dispenser).[24] By one estimate, electrically heated mats are the most effective spatial repellents, providing 56%–90.5% protection compared to 22%–87% protection from coils.[25]

Coils disperse active ingredients behind the burning tip of the coil as the critical temperature for volatilization is reached in each successive segment of the coil. As a result, the rate of dispersal is not dependent on temperature, but on burning rate. The burning rate can be affected by the composition of the coil, its shape (optimum diameter 0.36–0.42 cm), and surface coatings.[26] Typically, a coil burns at 2.1 g/h, lasting 7–8 hours.[19] Coating the outside of the coil with the active ingredient is actually more effective than integrating the chemical throughout the coil because the active ingredient in the outer layer is less subject to thermal decomposition. Synergists such as piperonyl butoxide and MGK 264 (*N*-octyl bicycloheptene dicarboximide) slow the knockdown effect but increase the percentage killed. Synergists are possibly less effective in coils than in sprays because the insect is exposed through the respiratory route.[26] People are also exposed through the respiratory route, and there is recent concern that the smoke from the coils can cause damage similar to that from second-hand cigarette smoke.[27]

There are very few reports that confirm the efficacy of anti-mosquito products out of doors. Jensen et al.,[28] in studies evaluating the performance of a variety of products including citronella candles and ultrasonic repellers, reported that only pyrethroid-based mosquito coils and deet products significantly reduced mosquito landing rates when compared with untreated controls.

Candles and torches are also available to consumers. Citronella, linalool, or geraniol is incorporated into the candle wax, or citronella into torch fuel, and is volatilized by the heat of combustion. Several repellent products, consisting of a lamp with heat-generating candles or butane cartridges and a repellent mat insert are currently registered by the U.S. EPA; all these products contain 21.97% allethrin as the active ingredient. An advantage of these latter products over coils and candles is that they do not smoke. A disadvantage is that they are far more expensive. At this writing, it appears that allethrin will lose its U.S. EPA registration soon and such products will have to shift to alternative active ingredients. Candles are also a popular format for the dispersal of citronella, presumably heating the active ingredient as the candle burns. A more sophisticated candle takes advantage of the greater activity of one stereoisomer of linalool [the (*S*)-(+) form] compared to another [the (*R*)-(–) form]. A different device uses propane to heat the same active ingredient.

Kerosene lamps have also been modified to heat a repellent active ingredient, either by suspending a small metal container with transfluthrin above the flame[25] or by mixing esbiothrin[29] or neem oil[30] directly into the kerosene fuel. These systems provided up to 96% protection against *C. quinquefasciatus*[25] and over 99% protection against *Anopheles culicifacies*.[29,30] These levels of protection appear to exceed those provided by mosquito coils or electric mats, and equal the levels of protection provided by topical repellents.

Another active-emission technology uses a fan to disperse vapors from a material that volatilizes under ambient conditions. The U.S. EPA has registered several products that consist of a cartridge containing either linalool or geraniol inserted into a stationary fan device that disperses the repellent vapors. Recently, several wearable personal repellent devices have received EPA registration. These devices consist of a small battery-powered fan and a repellent matrix treated with an active ingredient (metofluthrin, a pyrethroid; or a mixture of essential oils) that volatilizes at ambient temperatures. The devices create a cloud of mosquito-repellent vapor around the wearer.

Active ingredients normally used as topical repellents can also have a spatial effect when they are dispersed in the air. Early experiments showed that injecting dimethyl phthalate into an air curtain formed a barrier to house flies.[31] An arrangement in which deet was added to a filter behind a high-volume fan provided 88% protection from a population of *Anopheles punctipennis*, *Aedes vexans*, *Aedes stimulans*, and *Aedes trivittatus*.[32] Experiments in the laboratory with olfactometers have shown that dimethyl phthalate, ethyl hexanediol, dimethyl carbate, and deet can repel mosquitoes in the vapor phase.[33–37]

Passive-Emission Devices

Passive-emission devices contain an active ingredient sufficiently volatile under ambient conditions that they do not require heat, forced air, or other energy inputs to create sufficient concentrations of the repellent chemical. These products consist of materials such as paper, plastic, or vermiculite impregnated with the active ingredient. They are intended to release the chemical or chemicals into the air at as steady a rate as possible over a period useful to the consumer. Real protection from biting insects by these kinds of devices is clearly a big technical challenge, even though the public would very much like to use such a product. Wrist bands with various natural products had no significant effect on biting,[38] but deet-treated anklets provided good protection from *Anopheles gambiae* during 2 weeks of use for 2 hours per night.[39] Metofluthrin-containing products have come on the market recently. These products consist of various configurations of either paper or plastic impregnated with metofluthrin. In one product, metofluthrin is impregnated onto an accordion-like folded paper strip; the product is activated by removing the folded paper strip from its packaging, stretching it out, and hanging it at least 1.5 m above the ground. Recently, a new product intended to be used as an adhesive patch on clothing has been promoted as an important advancement in personal protection from malaria vectors. The active ingredients have been described as natural, but they have not been specified. The manufacturer claims that the patch will make a human host invisible to mosquitoes for up to 48 hours, acting as an attraction-inhibitor rather than as a repellent.

The public's desire for a product that could be used simply as an area repellent is reflected in the large number of compounds that were tried for this purpose either successfully or unsuccessfully.[4] The U.S. Army experimented with direct application of chemicals to the ground by spraying the topical repellent M-250 (60% ethyl hexanediol, 20% dimethyl phthalate, and 20% indalone), claiming 1 hour of relief from a mix of biting *Aedes* species in Alaska.[40] During World War II, the Soviets used xanthic disulfide, known as "K preparation," as an area repellent.[41] Deodall (terpenes, terpene alcohols, and terpene oxide) was a deodorant added to formulations of benzene hexachloride, an organochlorine insecticide no longer in use. Deodall was itself repellent to mosquitoes when used as a space spray.[42,43] Other materials that could be used as an area repellent included a wide range of plant-derived essential oils and pyrethrum sprayed indoors. An extensive series of experiments in the laboratory showed that the following materials placed near a mouse could provide 90% or better protection from *Aedes aegypti*: high naphthalene content petroleum oil fractions, citronellal, geraniol, pyrethrum, allethrin, *S*-bioallethrin, D-*trans*-allethrin, deet, and dimethyl phthalate.[44] The most effective material was a commercial product no longer available. This product was developed following the observation that a vermiculite preparation of DDT used as a prehatch insecticide for floodwater mosquitoes was also a repellent to biting mosquitoes. The solvent Velsicol AR50 used as a carrier for DDT was sufficient to produce the repellent effect. This solvent contained methylated napthalenes, petroleum distillate, and diesel fuel. The most active components were 1-methylnaphthalene and 2-methylnaphthalene.[45] Another use of naphthalene as an area repellent was by fishermen along the rivers of the midwestern United States. They were known[4] to scatter a few naphthalene-based mothballs around to get some relief from annoyingly abundant populations of *Aedes vexans*. This sort of home remedy has probably been in use for a very long time.

MODE OF ACTION

Over 45 years ago, Dethier[46] showed that chemicals elicit multiple actions and that insects respond to those actions through a variety of behaviors. He noted that if we were to take a closer look at modes of action, we could find a much more diverse set of terms for oriented movements of insects toward or away from a chemical source. As early as 1953, Muirhead-Thompson[47] concluded that chemicals could disrupt contact between humans and malaria-transmitting mosquitoes to stop disease transmission without killing the mosquitoes. Subsequent authors speculated that spatial repellents applied to house walls could have advantages over topical repellents on skin. In contrast to topical repellents, repellents designed for application on walls could be formulated for longer persistence and might even have a lower cost of production. There is evidence that true mode of action of DDT is as a spatial repellent, not as a toxicant. Grieco et al.[10] conducted laboratory and field studies using *Aedes aegypti* as a model species to quantify and accurately describe chemical actions of three insecticides (dieldrin, DDT, and alphacypermethrin). These insecticides elicited varying combinations of behavioral and toxic actions. These actions were defined in terms of the mosquito responses to the chemical. A toxic action produced knockdown or death after the mosquito made physical contact with the chemical. A contact irritant action stimulated directed movement away from the chemical source after the mosquito made physical contact. A spatial repellent action stimulated directed movement away from the chemical source without the mosquito making physical contact with the treated surface. The results of these studies showed that one of the three insecticides (dieldrin) was toxic, but had no repellent or irritant actions. Another (alphacypermethrin) had irritant and toxic actions, but had no repellent action. The third chemical (DDT) exhibited all three actions: repellency, irritancy, and toxicity. These studies further showed that both toxic (mortality) and sublethal (repellency) actions will produce a vector-free space; however, one is due to a direct killing action, whereas the other is not. Prior evidence, which supported their findings, had been largely ignored. Beginning in the 1940s, numerous observations were made on the ability of DDT

to create a vector-free space.[48] When DDT was sprayed on the interior wall surfaces of houses, there were essentially no mosquitoes to be found indoors, with malaria rates subsequently declining dramatically and vector populations reduced overall.[49–51] Those results are attributed primarily to the spatial repellent action of DDT (a significant and generally underrated property) and not to the toxic action alone.[52] Several studies supported the conclusion that the action of DDT was spatial repellency primarily, with contact excitation and toxicity as secondary and tertiary effects.[6,14,53–58]

Other studies[13] have reported that noncontact irritancy, spatial repellency, and noncontact disengagement, all describe behavioral end points resulting from exposure to spatial repellents. These active ingredients can induce mortality, deterrence, repellency, and reduce feeding by mosquitoes on humans. Although some mosquitoes die from exposure, spatial repellents are not generally efficient for reducing populations, especially outdoors. Pyrethroids used as spatial repellents probably act through two general modes of action.[4,16,18,19] First, a nonlethal effect resembling irritation, and possibly consisting of several physiological responses, causes the insect to avoid entering the area, to leave the area if it has already entered, and to fail to bite if it is already in position to do so. Second, the toxic action of the pyrethroids causes either reversible knockdown or death.

Thresholds exist for when and how insects respond to these chemical actions. These thresholds are governed by intrinsic factors related to the mode of action of the chemical and the insects' susceptibility, as well as by extrinsic factors that modify those effects. Among the extrinsic factors, the physical environment, humidity, air movement, and temperature can affect the volatility of the active ingredient. The dose-dependent order in which thresholds are exceeded determines whether the primary mode of chemical action is repellent, irritant, or toxicant. Some pyrethroids can produce excitorepellency with possible mortality as a result of the exposure.[14] Other pyrethroids with sufficiently high vapor phase concentration, for example, metofluthrin[59,60] and transfluthrin,[61] can result in a spatial repellent (barrier) effect regardless of knockdown and mortality of insects.

Wind tunnel tests using volunteers who exposed bare skin to laboratory-reared mosquitoes indicated that significant disruption to host finding occurred in the presence of airborne metofluthrin.[60] However, even when landing did occur, the majority of insects were still inhibited from biting. This sublethal effect resulted from pyrethroid induced neural hyperexcitation, which can occur at much lower doses than those required for insect knockdown and mortality. Winney[33] reported that female *Aedes aegypti* exposed for a few minutes to the smoke of pyrethrum coils, although not knocked down, still did not bite. These results suggest that actual rates of biting inhibition may be underestimated by tests based on knockdown or mortality.

The threshold level of chemoreception of an active ingredient by an insect should be useful for the determination of the distance at which a chemical affects the target insects. The concentration of an active ingredient volatilized from a point source will diminish over distance and will be affected by air movement. As a result, a chemical with equivalent volatility but lower chemoreception level will act as a spatial repellent at a greater distance than a chemical that the insect cannot detect at such a low concentration. Product development might be improved by determining the threshold concentrations of active ingredients under consideration, and then working on how to achieve those concentrations.

One way to measure noncontact irritancy is to use local houses or experimental huts fitted with exit and entry traps[18,62–65] for the comparison of the proportion of mosquitoes exiting untreated and treated structures. Using this approach, studies have shown an increased proportion of mosquitoes that exit earlier from huts with burning coils compared to huts that do not have coils.[18] This effect was proportional to the concentration of active ingredient generated in the huts. This indicates that the magnitude of irritancy might be dose dependent.[11]

Bernier et al.[3] extensively reviewed attraction-inhibitors. Included in the review were compounds produced by plants such as catnip oil (specifically the isomers of nepetalactone), geraniol, linalool, citronellol and citronellal, and human-produced compounds. In human-produced compounds, attraction-inhibition was observed for some carboxylic acids (saturated acid combinations of C_8–C_{10}

were found to prevent host location), aldehydes (e.g., nonanal), ketones (saturated ketones particularly in the C_7–C_{12} range inhibit attraction), and alcohols. Using a dual-port triple-cage olfactometer, Kline et al.[66] examined the impact of linalool, dehydrolinalool (3,7-dimethyl-6-octen-yl-3-ol), and deet on the host-seeking ability of laboratory-reared *Aedes aegypti*. Compared to dehydrolinalool and deet in competitive bioassays, linalool was the most potent inhibitor. An important finding of this work was that the release of linalool resulted in two observable effects on mosquito behavior. The first effect was that fewer mosquitoes in the cage were activated to flight during simultaneous release of attractant and linalool in the airstreams of separate ports of the dual-port olfactometer. This indicated that vapor phase linalool acted as an attraction-inhibitor by preventing some of the mosquitoes from detecting the normally attractive odors. The second observable effect was that mosquitoes activated to fly were less likely than controls to find hosts. This indicated that even though some mosquitoes could detect the presence of attractive odors, they were not capable of orienting toward and, thus, locating the odor source. Human inhalation of this compound is known to produce sedation. In addition, it has been shown to suppress the voltage-gated currents in newt olfactory receptor cells.[67] Birkett et al.[68] reported that linalool produced significant electroantennogram responses in four species of biting flies, and reduced the upwind (positive) anemotaxis in laboratory and wind tunnel studies. Linalool has two optically active isomers; researchers have found the (*S*)-(+)-enantiomer to be the better attraction-inhibitor.

GOALS AND LIMITATIONS OF AN AREA REPELLENT PRODUCT

The ultimate goal for the use of spatial repellents is to create a vector-free space. That space could be a picnic area to be occupied for a couple of hours or a rural hut occupied every night. The space might also be mobile, such as a car or a theoretical bubble surrounding a hiker. The purpose of any spatial repellent is to prevent biting, but it is worth considering subsets of that purpose that can influence the design of products. Starting with the least important, some people want a product that will relieve them from the worry of potential bites from either real or imagined arthropods. In this case, the effect is largely psychological and the effectiveness of the product is proportional to the consumer's perception. The effectiveness may be quite real and will reinforce the perception, but the technical demands on such a product are certain to be less stringent. A second purpose is to reduce the annoyance created by the presence of biting arthropods. In this case, the problem may be relatively severe so that any reduction in biting is considered significant. Although the consumer may continue to get some bites, use of the product may be viewed worthwhile because the annoyance was reduced from unbearable to bearable. Finally, an area repellent might be used to reduce or eliminate the risk from vector-borne pathogens. Even when the infection rate of the vector is low, the consumer should consider each bite a potential risk, implying that the tolerable biting rate is near zero. The area repellent may only supplement other personal protective measures, but this use would tend to place the most rigorous requirements on the product.

If spatial repellents do not kill vector insects, then there is concern that diversion of vectors from the protected area will actually increase the risk for people outside the protected area.[69] Accurate study of many aspects of the effects of an area repellent would be necessary to make a realistic appraisal of this risk. First, the actual number of insects diverted by the area repellent might be trivially small compared to the population exposed. Second, nonlethal effects on the insects might prevent biting even though they are not killed. Finally, the area in which there is partial protection from low concentrations of the area repellent would have to be determined.

A potential benefit of spatial repellents is a delayed or diminished development of insecticide resistance by minimizing the intensity of selection pressure from contact-mediated toxicity mechanisms. There could also be general benefits from more limited use of pesticides, if spatial repellents

made them less necessary.[70] The added long-term benefits of showing disease impact of spatial repellents include the discovery and development of new chemical active ingredients and/or new modes of action that target and exploit the normal patterns of vector behavior during host seeking. A better understanding of vector behavior in this context could stimulate innovative product development and enhance vector control. Among the possible behavioral modifications that would reduce disease risk for humans would be diversion of vectors to animal hosts, increases in the energy required to find hosts and oviposition sites, and increases to the time that an individual vector is exposed to the environment while seeking a host. Outdoors, vectors risk greater predation, physiological stressful environments, and excessive energy expenditure during host seeking, or identifying a resting or oviposition site.[57] Furthermore, vector populations that survive exposure to sublethal, spatial repellents may subsequently show permanent or semipermanent disruption of host-seeking and blood-feeding behaviors.[71] The reduction in host contact and feeding success could ultimately lead to reduced populations, especially of older females that are more likely to transmit pathogens.[57] Spatial repellents could be an important contributor to reduction of longevity, human contact, and insect infection rate that drive transmission of arthropod-borne pathogens.[72]

Spatial repellents have inherent limitations on both safety and efficacy. Unlike insecticides that might be applied at a distance, causing no exposure to people, spatial repellents must be applied in the surroundings of the consumer. With the exception of physical barriers, the consumer is necessarily exposed to whatever prevents the arthropod from biting. One result is that many products are not as safe indoors as they are outdoors. Efficacy is likely to be limited by several factors. The spatial repellent must work across a distance so that people at a greater distance from its source are likely to be at greater risk of bites. For chemicals, any air movement will tend to reduce efficacy. If the spatial repellent breaks the arthropod's chain of host location and identification, then there is the danger that a redundant biological system will enable some of the arthropods to reach their target and bite. Given these limitations on safe use and efficacy, it is unlikely that spatial repellents can ever provide the same level of protection as that provided by good topical repellent products. Nonetheless, spatial repellents are popular with the public because they can be used to protect one or more people without requiring skin application.

METHODS FOR EVALUATION

Until recently, no standardized evaluation methods existed for spatial repellents. However, the World Health Organization recently published[73] a document that provided guidance and described steps for standardizing laboratory testing, and for semifield and field evaluations of spatial repellent products. For laboratory testing of technical material, they recommend a modular high-throughput screening system.[74] The recommended system allows examination of toxic, contact irritant, and spatial repellent responses. This assay is typically performed under static airflow in a chemical hood. Although the guidelines recommend the use of a dual-port design, Y-tube olfactometer, a variety of suitable olfactometers can be used to measure host attraction and inhibition. The objective is to measure the ability of an active ingredient to inhibit mosquito attraction to a host. The Y-tube and similar olfactometers measure attraction to host odors in the absence and presence of the active ingredient.[3] Protective efficacy, measured by the difference in the inhibition of landing and feeding between treated groups and controls over time can be evaluated in free-flight testing rooms that measure at least 30 m^3. Measures of reduced entry and resting can also be tested using this arrangement.

Semifield studies were recommended to add significance to the results of laboratory efficacy studies and to test formulated products against free-flying populations of one or more target species. Semifield trials are conducted in screened enclosures (with or without experimental huts) using the release of well-characterized mosquito populations (usually from laboratory-reared

colonies). The advantage of using screened enclosures for these evaluations is that a known number of pathogen-free mosquitoes of fixed age and physiological status are used. Another advantage is that a known distance between the point in which the mosquitoes are released and the source of the chemical stimulus can be established, thus allowing estimation of the protective area. The use of a screened enclosure also allows the tests to be conducted in known local conditions at ambient temperature, light, humidity, and air movement. The enclosure needs to be sufficiently large to be representative of the area over which the spatial repellent product is intended for use.[75]

Field trials of formulated product are used to measure the personal protection offered by a spatial-repellent product against free-flying natural populations of mosquitoes. Efficacy is measured by comparing landing rates between treated subjects and controls. As the infectious status of natural populations is often unknown, special precautions (e.g., protective clothing) are often necessary to protect volunteers. The WHO guidelines clearly state that the participants' well-being must be assured and their autonomy respected. The inclusion and exclusion criteria for participation in a test, informed consent for risk of pathogen infection, pathogen detection and monitoring, as well as chemoprophylaxis and treatment should follow national guidelines, and the study protocol should be approved by the relevant research ethics committee in the country or institution in which the study is taking place. It is expected that if these guidelines are followed, it will create opportunities for industry, academia, and others to do more work toward the development of effective and safe area repellents based on behavioral modification of insects.[70]

Although not addressed by these WHO guidelines, some recent studies have been published on methods to measure airborne concentrations of the active ingredients.[76] It is hoped that these evaluations will lead to the determination of minimum effective concentrations required for creating vector-free spaces. Such studies could help developmental efforts by determining the length of time that an effective concentration remains in the air space and the distance at which the effective concentration can be detected. Achee et al.[76] developed methodologies for detecting and quantifying the air concentration of metofluthrin and DDT, under laboratory and field conditions. In the field experiments, technical grade DDT and metofluthrin coils were evaluated using experimental huts (50 m^3) in Thailand against laboratory-reared *Aedes aegypti*. The deterrent, knockdown, and 24-hour mortality responses of test populations on exposure to the coils and DDT-treated netting were quantified using a mark-release-recapture study design. In addition, the concentration of the chemicals was determined in the air near test mosquitoes. The metofluthrin coils reduced entry by 58% and achieved knockdown of 1.4% of the mosquitoes that entered. Chemical sampling under these conditions is challenging because meaningful results require sampling times of no more than an hour. Thermal desorption[77,78] can be used accurately with only an hour of sampling, compared to standard air collection methods that require 4 hours.

ENVIRONMENTAL FACTORS

The spatial activity of airborne active ingredients is affected by airflow, wind speed, temperature, and humidity within the treated space. The design of area repellent products must take into account the environmental conditions likely to occur where the products will be used. A faster air current causes more dispersion of a volatile chemical and lowers its concentration in the air, which degrades the effect of both attractants and repellents.[79,80] A study carried out in Tanzania showed reduced efficacy of emanators when used in houses with open eaves[81] compared to houses that did not have open eaves in Vietnam.[82] High temperature increases volatilization of active ingredient, which may improve efficacy but shorten the duration of the product. Those developing area repellents can be adjusted for some environmental effects by changing the concentration of active ingredient, size of surfaces from which the active ingredient volatilizes, and so forth.

CONCLUSIONS AND FUTURE DIRECTIONS

Although the idea of protecting people from vector-borne pathogens by altering the behavior of the vectors is not new, the concept has been underutilized. Spatial repellents could become an important part of integrated vector management, especially if they were used strategically to prevent infective bites that are not prevented by other methods. Those who actually lead efforts toward disease prevention are typically part of the public health community and not entomologists. As a result, addition of a single product to their armamentarium of tools for vector control is useful, but not sufficient to solve the disease problem. As important as developing spatial repellent products is to determine how best to use them for disease control. Before this can become a reality, Achee et al.[70] stated that there are hurdles to overcome; these hurdles include generation of epidemiological data proving efficacy convincingly for policy makers, identification of entomological end points that indicate sufficient effect to reduce disease, and improvement of chemical screening procedures to include behavioral as well as toxic effects on target vectors. Epidemiological studies are expensive, but some of the first attempts at high-quality, clinical-style studies have appeared in recent years and show some encouraging results about the use of repellents.[83] Innovative measurements of effects on insect behavior may emerge from basic studies of how chemical signals interact with receptors and the insect brain.[84] Chemical screening that includes behavioral tests is possible now, but high-throughput screening of repellency or other complicated behaviors may not be possible. In silico analysis of potential chemistries is a possible way to narrow the number of candidates for the more labor-intensive behavioral tests.[85,86]

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CHAPTER 13

Marketing for Efficacy, Acceptability, Safety, and Health

Larry Goodyer, Lawrence J. Feller, and Charles A. Feller

CONTENTS

INTRODUCTION

The global repellent market has been estimated at greater than $3 billion[1] and has experienced huge growth annually. In the United States in 2008, *USA Today* reported mosquito-fighting products were a $200 million-a-year market, which included sprays for the skin and biodegradable insecticides.[2]

Marketing strategies would benefit greatly from understanding of studies that examine bite-avoidance behavior and attitudes. Unfortunately, robust evidence is not currently available, limited

work has been conducted by the military[3] and by one of the authors of this chapter concerning travelers to malaria-endemic areas.[4–6] These latter studies will be referred to where applicable. The understanding of the ordinary person regarding different types of products and what to precisely expect from their use is also not well studied. For instance, anecdotally, the terms "insecticide" and "repellent" are often confused and used interchangeably. Some may not appreciate that a repellent might only reduce the chances of being bitten and environmental conditions, including particularly mosquito density, would reduce perceived effectiveness. Ideally then, where possible, formal market research strategies could be used before marketing any new product. Although this does not often appear to have been conducted, larger marketers do periodically employ firms to undertake such surveys, which would not be in the public domain because it is considered as confidential business information.

There is a very sizable range of products available for retail sale in a vast array of different formulations and presentations. If one just considers the *N*,*N*-diethyl-3-methylbenzamide (deet) based products on the market since the early 1950s, often within one company product range, there might be three, four, or more different strengths in a variety of presentations including sprays and pumps, lotions and creams, and impregnated materials (wrist bands, towelettes, and roll-ons). Other recently released synthetic and semisynthetic agents are increasingly available in a similar though often not quite as diverse a range of formulations, preparations, and packaging. Added to this is the assortment of "natural" products and devices such as electronic "mosquito buzzers" that are often of dubious efficacy. Generally, these other bite-avoidance products are not available in quite the same range. Within an increasing number of countries, there will be various insecticide clothing and gear treatments, heated vaporizers, and knockdown insect sprays.

These products are all found in a particularly wide diversity of retail environments such as

- Mass merchants/discount stores
- Supermarkets and groceries/convenience stores
- Pharmacies
- Outdoor stores and specialist travel outlets
- Travel clinics
- Online

Each of these outlets demands quite different marketing strategies. For instance, the health aspects discussed in this section would, in general, be most applicable to the travel clinics and pharmacies where there exists a one-on-one relationship between buyer and seller. Given the nature of their customer base (hikers, hunters, campers), the outdoor stores may be particularly useful for marketing tick bite–avoidance products including fabric treatments with repellent/insecticide preparations.

This review focuses on the largest market, which is for topically applied insect repellents. With such a diverse marketplace positioning, the marketing of any product is key to its success. The most useful place to start is matching the product, which, from the clients' perspective, is most likely to be the ideal purchase. This is summarized in Table 13.1 for topically applied mosquito repellents and in Table 13.2 for other products.

Table 13.1 Characteristics of the Ideal Mosquito Repellent

Characteristic	Comment
Efficacy	In even the highest mosquito densities, it will have a longevity of 6 hours before first bite
	Efficacy not affected by environmental condition, heat, humidity, sunlight, and so forth
Cost	Lowest cost and minimal amount of product required per application to achieve greatest affect
Acceptability	Good, cosmetically acceptable formulation; nongreasy, nonoily, and low odor
Safety	Highly favorable side-effect profile and minimal skin sensitization; hypoallergenic

Table 13.2 Ideal Characteristics of Some Other Bite Avoidance Products

Product	Comment
Insecticide clothing and gear treatment	Easy to treat clothing
	Does not damage or adversely affect fabrics; odorless
	Long time before retreatment
	Can launder clothing between treatments
Insecticide vaporizers	Mats or liquids last for several hours; can be used throughout the night
	Rapidly remove mosquitoes; repel, knockdown and/or kill
	Nonirritant and safe for asthmatics or those with lung disease
	Electric or gas operated
Mosquito netting	Ease of hanging for portable nets
	Mesh size for comfort of breathing
	Durable, ripstop material, nylon, polyester, natural, and synthetic blends
	Impregnated, long-lasting insecticide treatment

The approach will be defined to some extent by the type of market to which the product is aimed. These markets could encompass the following:

The traveler who may be visiting areas where there is a danger of arthropod-borne diseases. In this case, the marketing strategy would need to raise the awareness of such diseases and the importance of following the advice of health authorities regarding the most effective ways of avoiding contracting the disease. Such travelers will need to carry not only repellents but also the range of other devices and options that are available. This makes portability an important marketing tool. There could be an argument for promoting the highest concentration repellents (less would need to be applied and carried). However, in one study, there was no such correlation between repellent strength and quantity applied.

Those living in endemic countries where arthropod-borne diseases are a risk. In developed countries, examples include West Nile virus and some tick-borne diseases. Elsewhere, expatriate workers may be exposed to insect-borne diseases such as malaria, Leishmaniasis, and a range of arthropod-borne viruses (arbovirus), which may be an issue. The marketing of repellents will go hand in hand with the various public health methods being advocated locally. The transportation and ease of carrying a product will not necessarily be an issue here. The position regarding marketing to local populations in developing countries will not be discussed in this chapter.

Where there is no danger of arthropod infection and the goal is simply to stop the nuisance factor. Emphasizing the marketing for those on out-of-door activities can be particularly important for both leisure and business activities. Focusing on those situations where nuisance biting is recognized as the principle issue, a particular case is the midge bites experienced by those camping near lakes in some parts of the world—the most notorious in Scotland, Scandinavia, and the southeastern United States.

The following section will explore these themes in greater detail highlighting their importance when marketing products.

EFFICACY

A key aspect for marketing any repellent product will be claims made for efficacy. These claims will need to be described in any labeling of the product and also in the marketing material that is to be distributed. Actual claims on labeling will be made against the standards set by various regulatory organizations that regulate these types of products. In the United States, this is done

by the Environmental Protection Agency (EPA) at the national level and individual agencies at the state level. Canada's regulatory body is the Pest Management Regulatory Agency (PMRA) and one provincial entity in Ontario. The European Union (EU) Biocide regulations are currently in place within member countries and are scheduled to be fully enforced by 2014. The EU regulations are expected to encompass any claims that are made on product labeling.

For marketing purposes though, the most important aspect is not just the regulated requirements but the ability to clearly communicate the efficacy of the product to the lay person. It is appropriate at this point to reexamine what is meant as efficacy and how this might be best described.

The aim is that the repellent when applied to the skin is able to completely deter mosquitoes from biting for a claimed period, but this can be open to interpretation and has been used in a misleading way by some marketers. In reality, the key is often not if the product has repellent properties, for it surly does, but how much of the repellent is required to have the desired effect and how long will it last. A standard cage test can be used to identify the amount of repellent needed to be applied by estimating the ED_{90}. If this is considerably less than might usually be applied by an individual, then it can be assumed that an effective dose will normally be achieved in practice. From the author's own work, this is likely to be an average of 0.5–1.0 mg/cm^2 on the arms. Thus, providing that an effective dose of an order less than this is achieved, a reasonable repellent activity claim may be made.

Probably more important still for marketing purposes is how long the repellent is likely to last following the first application. From the work conducted on the kinetics of deet,[7] it is well established that the 95% protection level will be maintained, which is dependent on the concentration on the skin. This rise in protection time with applied dose follows first-order kinetics so that the time will reach a plateau for deet at a concentration of 2 mg/cm^2 active ingredient (a.i.). After that, the level of protection will fall of exponentially related to skin absorption and evaporation. In practice, it may mean that in conditions of low mosquito density, no bites are still experienced by the individual even though the level of protection has fallen by 50%. This has sometimes been used somewhat misleadingly by manufacturers to claim very long "repellence" times, when they are referring to data derived from cage or field tests beyond the time to first bite, that is, less than 95% protection.

Most of the information used to make such product claims will be derived from data obtained from either cage tests or controlled field trials. In reality, the user will be subjected to a range of environmental conditions that will greatly modify the efficacy and all that one can say with some certainty is that it is reasonable to expect a lowering of the number of bites compared to the situation if no repellent had been used. Principally these variables are as follows:

- Mosquito density, which is probably the most important variable. It may be unreasonable to expect any product to have an acceptable time to first bite no matter how much is applied in conditions of extremely high mosquito density.
- The actual a.i. concentration on skin. This may vary greatly and descriptions of "apply a small quantity" or "apply sparingly" for instance are liable to be interpreted as applying an amount of the product achieving a poor longevity of action. Without doubt, studies indicate that most people will apply suboptimal concentrations when following manufacturers labeling.
- Sweating, which will be dependent on the individual, environmental heat, and humidity.
- Physical abrasion from clothing and rubbing-away repellent.
- Individual variations in factors influencing attraction.
- Insect species. Some mosquito species encountered will require higher a.i. levels to achieve the same level of repellency. It would not be reasonable to expect the ordinary person to be aware of the various species they may encounter.

In reality, it is very difficult to accurately and precisely describe in a simple marketing message the degree of protection and for how long protection might last. To a point, the higher the a.i. concentration achieved, the longer the protection will last. Ideally, then marketing and labeling information should describe more precisely the amount to be applied (detailed spray application, symbol lengths

of cream, and milliliters for liquid formulations). This strategy has been used in the sunscreen industry and reinforced by dermatologists with some success on at least one military insect-repellent cream. Further statements could be added that describe reapplying the product should mosquitoes begin again to feed, or applying the product more regularly under certain conditions. In the United States, regulatory labeling requirements are increasingly requiring this.

One of the most effective ways of conveying an efficacy message is by comparing a non-deet product to deet, which is quite well recognized as being a universal or "gold" standard. Again, such comparisons have been abused. For instance, when marketers claim "as effective as deet," it may be they are comparing their product against a low-strength (≤15%) deet-containing product. The most powerful marketing method would be to substantiate the claim through head-to-head cage and field tests to produce a marketing statement that might read as follows:

> In standard trials comparing the same recommended amounts of our product to that of a product containing 50% deet, our product gave (the same, twice as long etc) complete protection.

In reality, there are few non-deet products that would actually be able to justify such claims and marketing of the deet-based products, particularly those with higher concentrations, could take advantage of this. Usually, however, the claims of "non-deet," "contains no deet," and so forth are made. These nonrepellency-related marketing claims are made in such a way as to invoke a negative or bad image of deet-containing products. Regulated products are not allowed to make these comparative claims in the United States. The so-called exempt repellents and insecticides, however, are allowed to make claims, and these products are typically of very low or no efficacy value.

For insecticide products, this concept of longevity of action is also important for marketing. In the case of any clothing, fabric and gear treatments, users will wish to purchase a product that does not require continuous retreatment, perhaps at least lasting the length of their trip away. In addition, they will wish to be able to frequently launder the product without retreatment. There are some pretreated clothing products on the market making claims of "lifetime" repellency, but the evidence base for these is difficult to substantiate as it is based on proprietary data. Insecticide vaporizers are also purchased for their ability to be used in a bedroom throughout the night. A more recent development for vaporizers with treated mats are "gas" operated heating diffusors, making them more portable from country to country where electrical sources vary and/or do not exist.

Finally, longevity of the insecticide applied to a bed net is very important when these are being used in malaria control strategies by the population in endemic countries, where the very long-lasting insecticide treatments are used. For occasional travelers who might only use a bed net for a single trip, this is probably of less importance.

ACCEPTABILITY

Apart from efficacy, probably, the overriding criterion for the user is the cosmetic acceptability—feel and smell—of the product. Indeed, it may be that the user will select the acceptability of the product over its true efficacy. There is an argument that the best product for all individuals is the one that they are happy to use regularly.

One study did show that acceptability is an important factor. Of the 150 participants who were planning to travel to a malaria-endemic area, when asked their opinion of the statement "Repellents are unpleasant to use," 47% agreed and 22% were unsure, which left only 31% disagreeing.[8]

Formulations to produce a more acceptable odor can be difficult to achieve as the usual additives could potentially act as attractants. Adding volatile oils that may have repellent properties may be a potential approach though again not all individuals would accept a particularly distinctive odor, and the ideal would be to achieve an odor that is fairly neutral and does not linger. The problem is confounded with the fact that a large percentage of the product may comprise the a.i. Thus, in recent years, the major deet synthesizers have developed "low-odor" technical products.

In terms of feel on the skin, deet has been described as somewhat oily if higher strength preparations are used. The range of presentations that could be marketed are described in the following sections.

Spray Formulations

One of the principle disadvantages of a spray is that individuals are often observed as not applying sufficient because of making too few actuations of the spray button. Certainly, less is applied by spray compared to lotions. In the application rate study, the amount applied as a spray was significantly less than that applied as a lotion to all body areas, for example, for the arms, 1.37 (SD 0.62) versus 1.97 mg/cm^2 (SD 0.85) ($p < .005$). Further, it is often observed that the individual will hold the spray distant to the body before actuating resulting in a mist of spray not reaching the skin and lost to the environment. Also, it is noted that some modern spray marketers use an actuator button that creates a fine mist. Although this is cosmetically appealing to the user, it results in more "drift" and less product contacting the skin. For this combination of reasons, pressurized applicators are the least efficient.

It is sometimes also advised that the spray is applied to the hands and then rubbed over the body, particularly important if using anywhere near the face area. This procedure, for facial applications, is now enforced by the U.S. EPA. In fact, some labels recommend applying to the back of the hand first as there would be less of a tendency to want to wash the hands immediately after use. However, it is likely that individuals will purchase a spray over a lotion for the perceived reason that it could be applied to the skin without involving rubbing over with the hands.

Other Formulations

Anecdotally, it is the spray formulations that appear to be most popular in the United Kingdom, whereas in the United States, lotions seem to be preferred. With the advent of the "cosmetically" preferred sprays, however, the U.S. market may be shifting at this time. Another popular alternative that may give a good application rate is the roll-on preparation, though it has not been tested in recent years. There are surprisingly few of these formulations on the market: Net Effect Roll-On! Insect Repellent (United States), XPEL Mosquito & Insect Repellent Roll-On (United Kingdom), and Mosquito Milk Tropical Insect Repellent Roll-On (United Kingdom) are three of the few that contain deet. Natural roll-ons include Go 'Way All Natural Insect Repellent, Mosi-guard Natural Insect Repellent, and OnGuard Natural Insect Repellent.

Convenient to use are repellent impregnated wipes as these can be carried in individually wrapped sachets, which could be rubbed over the exposed skin in a well-targeted manner. However, it is unlikely that they will give a reasonable application concentration as the amount of liquid deposited would be much lower than other formulations. There are very few of these towelette products found in the market today.

Several preparations containing both repellent and sunscreen are also available on the market. The idea is that the product could be used when there are daytime biting insects present. Although an attractive concept for the consumer, there are many drawbacks in terms of efficacy. It is well established that deet will reduce the efficacy of certain sunscreen ingredients, so careful formulation and testing is important. Mosquitoes tend to bite during the cooler parts of the day when sunscreens are unlikely to be used. In addition, the reapplication times of the repellent may not coincide with the times the sunscreen is also needed, and the combination preparations are unlikely to contain high concentrations of repellent a.i. necessary to give optimal efficacy. The U.S. EPA has been developing an approach, in conjunction with the U.S. FDA, for the use and labeling of these products.

In conclusion, it is reasonable to produce a range of formulations based on the principle that there will be an individual preference by consumers. There is a need for more user preference data

to be available in the public domain, which may allow manufacturers to establish those that would prove to be the most popular and the reasons for their selection. Also, the U.S. EPA has recently published survey results that suggest a harmonized symbol system on repellent products that easily relate the insect repelled and for how long to the user.[9] Whether or not this labeling scheme will be introduced has not yet been announced.

SAFETY AND TOXICITY

This aspect of marketing repellents is of importance when reassuring the public of the safety profile of the products. Any topically applied product is likely to exhibit a certain level of skin hypersensitive reactions and this should be tested thoroughly in any premarketing work, often, but not always, being a requirement of the regulatory authority. It is prudent to always describe on the labeling some warning to apply the repellent to a small part of the skin during first use to determine any likely reactions. Repellent formulations may well be irritating to mucus membranes and the eyes, so warnings against avoiding application to these parts of the body are common. It is also almost universal to describe that the product should not be swallowed.

The most controversial aspect is the likelihood of any systemic toxicity as a result of topical application. As mentioned earlier, this is often used as a marketing tool where a marketer might claim that their own product is "safer" than that of deet. This section therefore focuses on the safety profile of deet to review the evidence base behind such marketing claims and why there is a tendency among the public to perceive deet as having an unfavorable safety profile.

There are a few available studies that have examined the public's true attitude toward the potential safety and toxicity issues. The study on those visiting malaria-endemic areas actually identified a relatively favorable attitude to repellents and the majority of subjects were using a deet-based preparation; only 18% agreed with the statement that repellents were toxic or bad for the skin, 30% disagreed, and 52% were unsure.

Deet

There have been several reviews concerning the safety of deet[10,11] and they have attested to its generally acceptable safety profile in normal use, supporting the recommendation that it is the repellent of choice when visiting areas of endemic arthropod-borne diseases, particularly those transmitted by mosquitoes.

Deet has been widely used worldwide since the 1950s and it is not surprising that several case reports linking deet to a variety of adverse events have been reported in the literature. Experience in a large population of users of the other available products is far more limited and case reports are few in number, so in this respect, the evidence base for safety of non-deet products could be defined as more limited.

The conclusions from an early short report in 1998 remain largely unchanged in that deet has a good safety profile in normal use. A large-scale review of reports to the U.S. Poison Control Centers in 1985–1989[12] and another in 1993–1997,[13] resulted in very few adverse events from dermal application. Most symptomatic exposures were as a result of accidental ocular application or inhalation.

Systemic Toxicity in Adults

Topically applied deet is absorbed into the circulation at about 8%–15% of the applied dose.[14] It is possible that ethanol formulations result in higher dermal absorption.[15] Elimination is rapid and near complete within 12–24 hours. Some animal studies have indicated neurotoxicity but are difficult to translate into normal dosage rates used by consumers.

Only four reports of adverse systemic effects, three central nervous system and one cardiovascular system, in adults as a result of topical use could be identified in the literature. In three cases, these related to very heavy and unusual use of repellent or alternative explanations of the symptoms could be offered. In a fourth more recent case of encephalopathy, the authors suggested a causal link to the measured serum concentrations.[16]

Systemic Toxicity in Children

This is a contentious issue and has led to inconsistent recommendations regarding the use of deet in young children. For instance, the Canadian PMRA view is that no more than 10% deet is applied to children,[17] whereas in the United Kingdom up to 50% is recommended if visiting malaria-endemic areas.[18] The Canadian recommendations have been made through an interpretation of animal data. Encephalopathy appears to be the most reported potential adverse effect accounting for about 12 cases since the early 1960s.[19] In many of these cases, either an alternative explanation for the encephalopathy can be identified or there may have been inappropriate application rates. Overall, it can be concluded that causation between the few reported cases of encephalopathy in children and the topical use of deet cannot be supported by a good evidence base.[19] Following a congressionally mandated Data Call-In in the United States, the EPA found no data indicating a need to restrict the use of deet for children or adults. All products must incorporate a series of 14 statements informing the consumer on the method of application, special precautions for children, and directions for medical attention.

Local Adverse Effects

Local dermal reactions to deet have been reported as being responsible for the majority of symptomatic exposures in the Poison Center study. Apart from local irritation, cases in the literature report forms of allergic contact dermatitis,[20] although these are likely to be rare. There was a series of early case reports of a more severe local contact dermatitis in servicemen who had applied deet before retiring and the exposed skin was occluded in the antecubital fossa.[21]

Use in Pregnancy

Several reviews and studies have failed to identify any potential for deet to cause harm to the developing fetus or a breast-fed infant. The safety of 20% deet in the second and third trimester was established by a study involving 897 subjects and only trace amounts of deet could be detected in 8% of the cord samples.[22] Human data for the first trimester are lacking, but animal work does not indicate any teratogenic effect.[23]

Interactions of Deet with Other Substances

Sunscreens

As discussed earlier, there is evidence that deet can reduce the sun protection factor value of sunscreens and in one study by a mean of 33%.[24] Another study also confirmed that sunscreens do not reduce the efficacy of deet.[25] Work on animals and in vitro studies on human skin suggest that the concurrent use of deet and sunscreens results in the enhanced absorption of both deet and the a.i. in the sunscreen.[26] One researcher has suggested that the effect may be reduced by applying the sunscreen before deet and, consequently, at least one marketer has advised the same when using their products. One study did identify that some loss[27] of deet activity results when applied beneath a sunscreen, but the true significance of the affect in use involving higher strength deet products

warrants further work. Only one clinical case report could be identified, which suggested toxicity may have resulted from overuse of a combined product. The U.S. EPA continues to investigate these combinations.

Permethrin

It has been suggested that the combined use of permethrin and deet can result in neurological damage.[28] The evidence is based on animal models that appear to use doses of permethrin and deet much higher than those in normal use and there are no human studies that suggest such a risk. Extensive research of permethrin-treated fabrics and garments suggests that the molecule chemically binds to fibers with less than 2% migration to skin followed by rapid metabolism.[29] The overwhelming evidence, therefore, strongly supports the use of permethrin-treated garments in combination with deet use on exposed skin for prevention of insect bites.

Icaridin, IR 3535, and p-Menthane-3,8-Diol

In comparison to deet, very little surveillance work or studies, other than those in animals, have been conducted to determine the true safety profile of the other leading market repellents. Their use has not been nearly as extensive as that of deet, and some such as 2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester known as icaridin, picaridin, or Bayrepel, have only recently been marketed. It is therefore not surprising that few reports of potential toxicity can be found in the literature.

The biopesticide, IR3535 (3-(*N*-butyl-*N*-acetyl)-aminopropionic acid ethyl ester) has been in use for the longest of the three having been introduced in Europe in 1979 and the United States in 1999. No case studies could be identified concerning reported toxicity, although it is difficult to gauge the extent to which it has been formulated into various products. Further, very little in terms of kinetics and toxicity profile is available in the literature. A similar lack of data applies to *p*-menthane-3,8-diol.

Bayer, the manufacturers of icaridin, has provided data indicating a good safety profile in that little is absorbed through the human dermis. One confirmed report of contact dermatitis from icaridin in a particular formulation was identified.[30]

HEALTH

A very important market is of those individuals who are purchasing repellents to reduce the risk of arthropod-borne diseases. Commonly, these would be travelers (tourists) visiting, as well as workers on long-term assignments (missionaries, oil field development, etc.), the tropics where mosquito-borne diseases in particular are a major issue. There are also some conditions expected in temperate climates where the local population may also use repellents, such as to avoid tick-borne diseases or West Nile virus. This section examines several arthropod-borne diseases and defines the place of repellents in reducing the risks of contracting such infections. This type of information is useful when marketing any repellent as it not only advertises the importance of using the product, but is also an opportunity to educate the public concerning such issues. The more the risks of contracting arthropod-borne diseases in certain situations are emphasized, the more likely an individual will be to purchase and use an appropriate product. As mentioned earlier, the marketing of repellents to local populations in the tropics to avoid diseases such as malaria will not be discussed here. There are also a large range of arthropod-borne conditions that are important in the tropics, but rarely an issue for travelers (filarial infections), which will not be discussed.

Malaria

Of all tropical diseases, this poses the greatest risk to the traveling public. Most authorities recommend bite-avoidance measures as part of their strategies to reduce the risks. For instance, in the United Kingdom, there are clear national guidelines regarding the use of bite avoidance in travelers visiting malaria-endemic areas.[18] It is also common for many countries to advocate not using chemoprophylaxis when visiting countries where contracting malaria is of a low risk, but to rely instead on bite-avoidance measures alone. One country, Kenya, enjoys over 200,000 tourists a year. Collectively, there are hundreds of thousands of visitors to these destinations annually. This therefore represents a large market for all of the related bite-avoidance products.

As it is, the *Anopheles* mosquito that transmits malaria, the various bite-avoidance strategies are designed to reduce biting from this "night time" feeder. The range of bite-avoidance advice is shown in Table 13.3. It is useful to include this type of information when marketing a repellent product as it raises awareness of the importance of these diseases to travelers as well as presenting an ethical and professional image by the manufacturer.

Dengue

This disease, which is an arboviral infection, has quite a similar distribution to malaria thus, a large number of travelers will also be at risk. In recent years, there have been some very large-scale epidemics particularly in Asia, South America, and parts of Oceania (geographical region

Table 13.3 Bite Avoidance Advice to Travelers to Malaria Endemic Areas

Advice	Discussion
Apply insect repellent to exposed skin particularly between dusk and dawn	Ideally, a high-strength deet concentration is the first choice (≥50(), though see the discussion in the text. The choice of product will also depend on local regulatory policy. Applying as the sun goes down is the general rule; however, travelers are also often unaware of the mosquito activity in the early hours just after sunrise
Cover up exposed skin as much as possible when going out between dusk and dawn. Wear loose fitting, long sleeves, and trousers. Wear socks to protect the ankles	The limitation to such advice is that it is somewhat incompatible with the tropical climate. Certainly many individuals will wish to wear short-sleeved clothing. If out at night and stationary, burning insecticide impregnated coils or portable vaporizers can also be useful to deter mosquitoes
Consider treating clothing with an insecticide	Mosquitoes can bite through certain types of clothing, particularly if tight-fitting cotton socks would be an example. Wearing insecticide-treated clothing helps avoid such problems and has been generally shown to reduce the level of biting when used in combination with a skin applied repellent.[31] When anticipating visiting areas of higher mosquito activity, travelers should pretreat clothing and gear with commercially available permethrin products. Pretreated clothings are also now widely available
Clear the room off mosquitoes before going to bed and keep the room clear as much as possible	This is achieved by a knockdown spray or more popularly by an insecticide plug in electronic or gas operated vaporizers. Typically, vaporizer mats will last for 4 hours and liquid filled much longer. The room should be well screened and the vaporizer should be used throughout the night
Use an insecticide-treated bed net while sleeping unless staying in a well-sealed and air-conditioned room	Bed nets for travelers will be most used by those backpacking and staying in budget accommodation. Portability of the net as well as suitable hanging devices is an issue. There are a range of bed nets with hanging materials available, designed specifically for the traveler

Table 13.4 Some Arboviral Diseases Transmitted by the Mosquito

Disease	Discussion
Chikungunya	An arbovirus similar to dengue and also spread by *Aedes* and also the *Culex* mosquito. Daytime bite-avoidance measures would need to be taken. *Culex* mosquitoes are present in part of Asia and Oceania. Epidemics have sometimes resulted in quite a large incidence in travelers. Through the Asian tiger mosquito (*Aedes albopictus*), this could become endemic in New York and other areas of the United States within a few years
Yellow fever	Present in parts of South America and Africa, spread by *Aedes aegypti* mosquito. The vaccine is highly effective, but may be contraindicated in some people such as those who are pregnant or elderly, in which case bite avoidance is essential
Japanese encephalitis	Found in parts of Asia particularly rural areas. Quite a seasonal incidence and spread by the *Culex* species. A highly effective vaccine is available
West Nile fever	This has become an important disease in the United States and Canada in recent years. The elderly are at most risk of severe disease. Repellents would be needed when a risk alert has been given
Ross River virus	Sometimes outbreaks are reported in Australia. Spread by both *Aedes* and *Culex* species. Bite avoidance is the only available preventive measure

Source: Adapted from Goodyer, L.I., *Travel Medicine for Health Professionals*, Pharmaceutical Press, London, 2004.

comprising the Pacific islands of Micronesia, Melanesia, Polynesia, Australia, and the Malay Archipelago), which have caused a huge number of deaths. In travelers for immunological reasons that will not be discussed here, death from the complications of dengue is very rare though it can be extremely debilitating. The number of notified cases of dengue in returned travelers only amounts to a few hundred annually. However, it is believed that because of the variability of symptoms, there are a large number of cases that are undetected.

There is no prophylactic medication or vaccine available to prevent dengue, so bite avoidance is the only method to reduce the risks. Since dengue is transmitted by *Aedes aegypti* and *Aedes albopictus*, which are "daytime feeders," repellents become the main strategy in bite prevention. In addition, the advice regarding the covering up of exposed skin before dusk is not likely to be followed in hot climates. It may also be unreasonable to expect travelers to be applying repellent throughout the daylight hours. It may therefore be more practical to advise paying particular attention during the cooler parts of the day, such as the morning and later afternoon, when mosquito activity might be higher. In many regions, malaria will be present and it is also likely that mosquito species, including indoor feeders, will be present so that the precautions described in Table 13.4 will need to be followed.

Other Mosquito-Borne Arboviral Diseases

There is a vast range of arboviral diseases transmitted by mosquitoes, and a few of those that are important to the travelers or in areas outside the tropics are presented in Table 13.4 along with a short discussion. Many of these also occur where other arthropod-borne diseases are present so that a repellent would usually be necessary.

Tick-Borne Diseases

There are three principle types of tick-borne diseases for which the use of repellents will play a useful part in reducing the risks of contracting an infection: rickettsial/spotted fevers also known as

typhus, Borrelia and principally Lyme disease, and the viral infection tick-borne encephalitis. All of these diseases are transmitted by the *Ixodes* (hard) ticks that live in the grasses of the woodland areas. It is therefore, walkers, hikers, surveyors, hunters, and the like who tend to pick up such ticks usually found attached to areas around the ankles and feet. The relapsing fevers are transmitted by the Argasid (soft) ticks. The points below concerning these conditions will be of value when marketing the products used by the public in raising health awareness.

Lyme disease is a zoonosis with intermediary vectors of several mammals including rodents and deer. It is found across much of Europe and North America with peak times from March to June. It is a good advice that people should remove a tick as quickly as possible without squeezing it so as not to inject further saliva from the tick, which may be carrying the infectious pathogen. In fact, in the case of Lyme borreliosis, the pathogen must travel from the midgut of the tick to its salivary gland during feeding, so contracting the disease is unlikely if the tick is removed within 24 hours.

Tick-borne encephalitis (TBE) is found as a broad band from Europe to Siberia and is also present in Japan and parts of Asia. In Europe, Austria has one of the highest incidences. Those expecting to be trekking and walking in forested areas of endemic countries are often advised to receive the TBE vaccine. The virus in the saliva of the tick multiples rapidly during feeding so early removal of the tick would not be of great benefit.

Rickettsia spp. are extremely widespread with different forms being present in a variety of regions. These range from the relatively mild African tick bite fever to the sometimes life threatening Rocky Mountain spotted fever in the United States.

As discussed earlier, insecticide-treated clothing is among the best strategy for avoiding tick bites and this is one of the principle marketed reasons for insecticide clothing treatments. The advice is usually to treat both socks and trousers. In addition, trousers should be tucked into the socks when out walking. The deet products can also claim some efficacy against ticks and it is likely that the use of both could be advantageous.

Arthropod-Borne Diseases Spread by Other Flying Insects

There are several types of flies that can also transmit diseases but these are quite rare in travelers. For completeness, they are described in Table 13.5. For all, there is very little evidence of the effectiveness of the available repellents that are used primarily against mosquitoes. In general, deet does appear less effective at deterring flies compared to mosquitoes. Marketers have sometimes been able to identify niche markets for fly-repellent formulations containing deet, MGK264 (a synergist) and MGK326 (a fly repellent).

Table 13.5 Some Diseases Spread by Other Flying Insects

Disease	Vector	Comments
African sleeping sickness (Trypanosomiasis)	Tsetse fly	The bite of the tsetse fly can be very painful. The disease is treatable, but diagnosis in travelers can be missed
River blindness (Onchocerciasis)	Black fly	The disease is more likely to be seen in travelers living and working for a period in some parts of Africa. The bite of the black fly can cause quite a prolonged and severe skin reaction
Leishmaniasis	Sand fly	Found most commonly in part of North Africa and the Middle East. The sand fly bites at night and is quite low flying. The best strategy for avoidance is insecticide-treated bed netting rather than the use of repellents

Source: Adapted from Goodyer, L.I., *Travel Medicine for Health Professionals*, Pharmaceutical Press, London, 2004.

SUMMARY AND CONCLUSION

For those marketing insect repellents, a broad range of issues should be considered to achieve the best market penetration. This can be summarized as follows:

- Acceptability to the user in terms of cosmetic properties, delivery device, ease of use, and cost
- Whether marketing the product to avoid nuisance, biting, and/or reduce the risk of arthropod-borne diseases
- Clearly showing the efficacy and safety profile of the repellent in marketing and labeling, in particular, when compared to other marketed products

Where available, evidence-based data should inform this process, but in the areas related to bite avoidance, behavior, and attitude more work is needed. Finally, it should be remembered that promoting the increased use of repellents has important health implications that should not be underestimated.

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CHAPTER 14

Klun & Debboun Modules: Uses and Data Analysis

Jerome A. Klun and Matt Kramer

CONTENTS

IN VIVO K & D MODULE BIOASSAY

The first Klun and Debboun module (K & D module) was designed for in vivo experimental use on humans[1] to evaluate the mosquito-feeding deterrent efficacy of chemicals that were toxicologically safe for application on the skin of humans. Impetus for development of this bioassay system resulted from problems with experimental techniques developed and used on humans between 1983 and 1992. These methods often required lengthy periods for observation and reducing the number of replications. The physical designs of the apparatus, where mosquitoes comingled in a common area and might have switched between feeding areas, made the data multinomial and induced correlations, making them more difficult to correctly analyze.

Details of the K & D module are shown in Figure 14.1. The module was made of acrylic plastic and with six 100-cm^3 individual cells. Each cell had a stopper-access hole for transfer of mosquitoes into the cell and a sliding bottom door (Figure 14.1, F). The module was designed to be used on a human thigh. It was designed concave to conform to the curvature of a human thigh (Figure 14.1, End view). A separate length of acrylic plastic, identical to the module's base with six rectangular

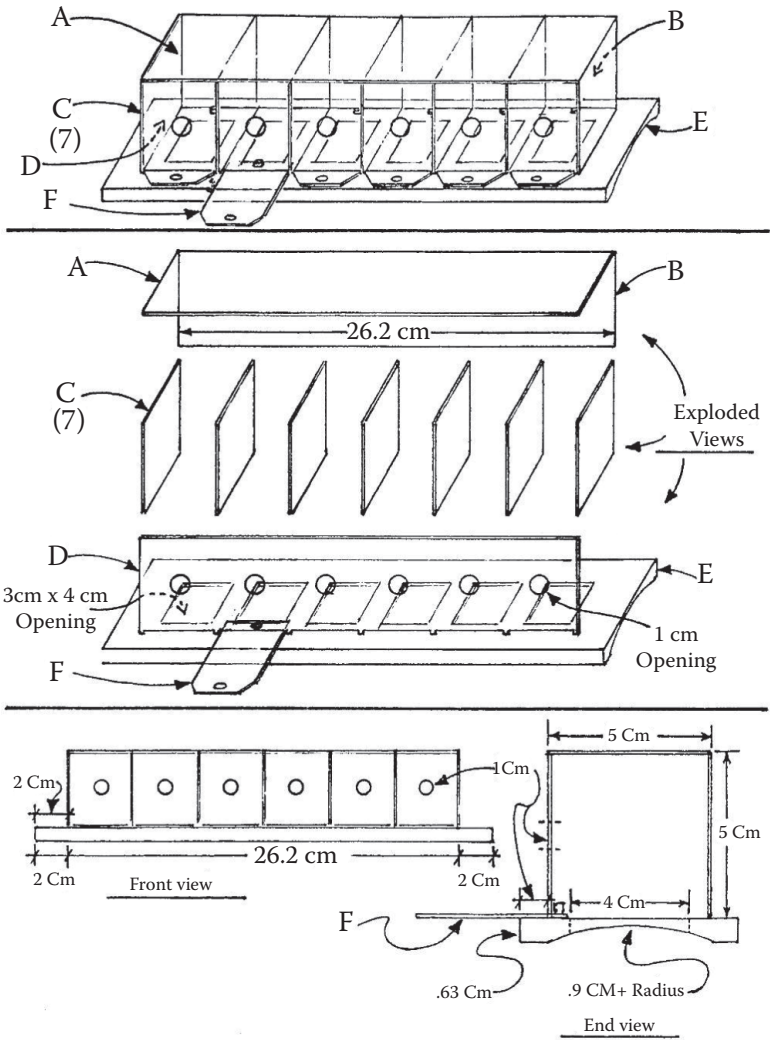

Figure 14.1 Line drawing of the K & D module illustrating components and dimensions from various viewpoints.

openings (Figure 14.1, E), was used as a template to mark areas of skin to be treated with test chemicals. High-quality modules for use on humans and the base template shown in Figure 14.2a are available commercially from Precision Plastics (Beltsville, MD).

Early on, Coleman et al.[2] evaluated the performance of the *N*,*N*-diethyl-3-methylbenzamide (deet), 1-(3-cyclohexen-1-ylcarbonyl)-2-methylpiperidine (AI3-37220), and 1-(3-cyclohen-1-ylcarbonyl) piperidine (AI3-35765) on humans against *Anopheles stephensi* Liston using the American Society for Testing and Materials Standard E951-8 plastic cage bioassay.[3,4] The plastic cage was open and rectangular (18 cm × 5 cm × 4 cm = 360 cm^3) with a screened top and five circular holes (29 mm diameter) in the floor, and a slide that permitted opening and closing of the holes (Figure 14.2b). In practice, a template matching the floor openings was used to mark and randomly treat circular areas on a volunteer's forearm. One area served as control and the remaining four were randomly treated with different doses of repellent chemical. The plastic cage, filled with 20 mated female mosquitoes (mosquito density equivalent to 0.05 female/cm^3), was secured over the treated forearm skin areas, and the floor slide was pulled out to expose all mosquitoes to all five skin treatments. The number of insects biting on each treatment site within the plastic cage was

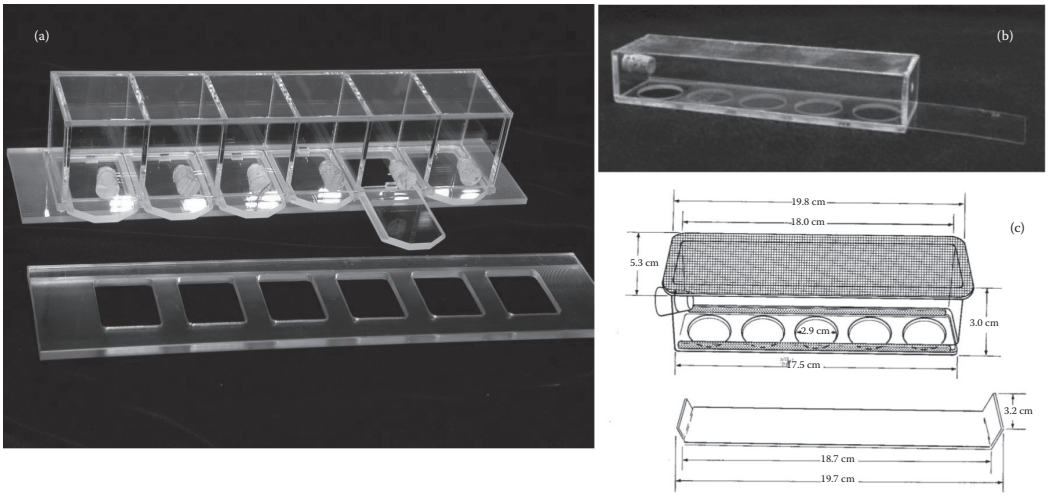

Figure 14. 2 (a) K & D module made of acrylic plastic. Template for marking of volunteer's thigh areas for treatments is shown in front of the module. Modules constructed with acrylic plastic are durable and washable in automatic washers. The module and template can be obtained from Precision Plastics (Beltsville, MD). (b) American Society for Testing and Materials Standard E951-8 plastic cage design. (c) Modified E951-8 cage design (from Gupta et al.[5]) for autoclave cleaning, and providing dimensions (same as for the E951-8 plastic cage design).

recorded during each minute of a 5-minute test period. This design was modified by Gupta et al.[5] so that it could be easily autoclaved between uses, though proportions were not changed (Figure 14.2c, from Gupta et al.[5]).

In the very first test of its bioassay power, the in vivo K & D module was used to evaluate the three compounds that Coleman et al.[2] had previously tested in vivo against *Anopheles stephensi*. In the testing, each of the four module cells was charged with five mosquitoes making for a mosquito density equivalent to the density Coleman et al.[2] used in their E951-8 plastic cage tests. Notably, all K & D tests conducted after 2000 used an insect density of five mosquitoes/cell. Data obtained with the in vivo K & D module showed that deet and AI3-37220 performed equally well against the mosquitoes and that AI3-35765 was the least effective. Data gathered by Coleman et al.[2] supported the same conclusion, but they required 36 replicates for their evaluation of the 3 compounds. In contrast, the K & D module provided the same quantitative results with 18 replicates. The improved bioassay efficiency over the open plastic cage (which simultaneously exposed all mosquitoes to all treatments) was attributed to the K & D module design that isolated the mosquitoes and dosage treatments in a replicate, eliminating the need for each mosquito to assess all treatments or doses available in the open cage, and reducing sampling variability. Isolated cells of the K & D module not only eliminated the need for mosquitoes to choose among treatments or doses but also permitted designs that better isolate the sources of variation. For example, one can simultaneously evaluate several species against an individual repellent at a single dose or the responses of several species to several candidate-repellent chemicals or doses. The K & D module assay system, either in vivo or in vitro, was designed to be utilized with chemicals having low vapor pressures, similar to that of deet. Use of candidate chemicals with higher vapor pressures could result in leakage between cells.

Rutledge and Gupta[6] referred to the isolated cells of the K & D module design as the "no-choice test module" and advocated that test systems should be designed, or redesigned, to function in the no-choice mode to provide results with less variability. Furthermore, in contrast with the Standard E951-8 plastic cage, which was positioned on the forearm of a volunteer and permitted two replicates

to be tested within one volunteer, the K & D module can be used on the outer, top, and inner thigh of each leg of a volunteer, permitting six replicates to be tested in one sitting of each volunteer. This gave the K & D module more replicated units than the Standard E951-8 plastic cage, though each unit had fewer mosquitoes. In general, this is an asset: experimental design recommendations are to have smaller and more numerous blocks.[7]

Between 2000 and 2003, the in vivo K & D module was successfully used to study the influence of optically active chemicals (antipodes) against mosquito blood-feeding behavior.[8,9] The optically active compounds were available in very limited amounts (5–10 g each), but testing of these antipodes was possible because of the sensitivity of the K & D assay that required only micrograms per square centimeter skin doses of the precious compound applied to the volunteers' skin. The K & D module was used in studies to demonstrate the importance of optical forms in mosquito-repellent activity and led to the patenting of a most potent optical form of the repellent chemical (1*S*, 2*S´*)-2-methylpiperidinyl-3-cyclohexen-1-carboxamide (SS220).[10] It was then used in a study to develop an efficient organic chemical synthetic method for preparation of SS220, and to evaluate the compound's performance as a repellent compared to the benchmark-repellent compounds, deet and hydroxyethylbutyl piperidine carboxylate (Picaridin), against two species of mosquitoes that are important vectors of yellow fever, dengue, and malaria.[11] This work was part of a broader objective to develop a new, effective, and safe repellent product for use against arthropods that are disease vectors. By using the in vivo K & D module with human volunteers, it was demonstrated that the protection afforded by deet, Picaridin, and SS220 against *Aedes aegypti* (Linnaeus), *Anopheles stephensi*, and *Phlebotomus papatasi* Scopoli bites was due to repellent and deterrent effects[12] (according to the standard terms developed by Dethier et al.[13] to describe chemicals in terms of the behavioral response they evoke). Readers are encouraged to review this publication[12] in detail because the article reveals several innovative ways that the in vivo K & D module can be used to reveal the behavioral mode of action of compounds against mosquitoes and sand fly blood feeding.

Figure 14.3 depicts the step-by-step use of an in vivo K & D module on a human volunteer in a quantitative experiment designed to evaluate the dose × response of *Aedes aegypti* to SS220, deet, and Picaridin.[11] Volunteers who participated in the bioassays were not selected for participation by using any prescribed volunteer-selection characteristics. A person's willingness to participate by offering their skin for exposure to chemical treatment and insect bites was the only requirement for a volunteer's involvement in experiments. Bioassays were always conducted without any prescribed prebioassay treatment or conditioning of a volunteer's skin. We thought that such skin conditioning might bias test results. Volunteers were always required to sign a consent form and verify that they were not susceptible to allergic reactions from insect bites. Before the initiation of in vivo K & D module testing was undertaken, it was verified that all planned test procedures conformed to the established National Institute of Health guidelines for tests involving humans, and also complied with the approved protocols established by a local Human-Use Research Committee. In addition, it was solidly confirmed that the compounds applied to volunteers had abundant chemical safety databases that allowed dermal application to humans. The source and chemical purity of the compounds used were also firmly established. This quality control step is absolutely essential because the validity, integrity, and meaningfulness of bioassay results are dependent on the verified identity and purity of the chemicals being tested.

The in vivo K & D module human bioassay method could be usefully applied in final stages of product development and marketing of a chemical that has cleared Environmental Protection Agency toxicology testing requirements, and where developers might wish to validate a product's performance for protection of humans against important mosquito and sand fly vectors of human diseases before it is released for public use. Moreover, there is good published evidence[8–12] that the in vivo K & D module–based bioassay method is robust, practical, and useful in research and development of new effective repellent chemical products.

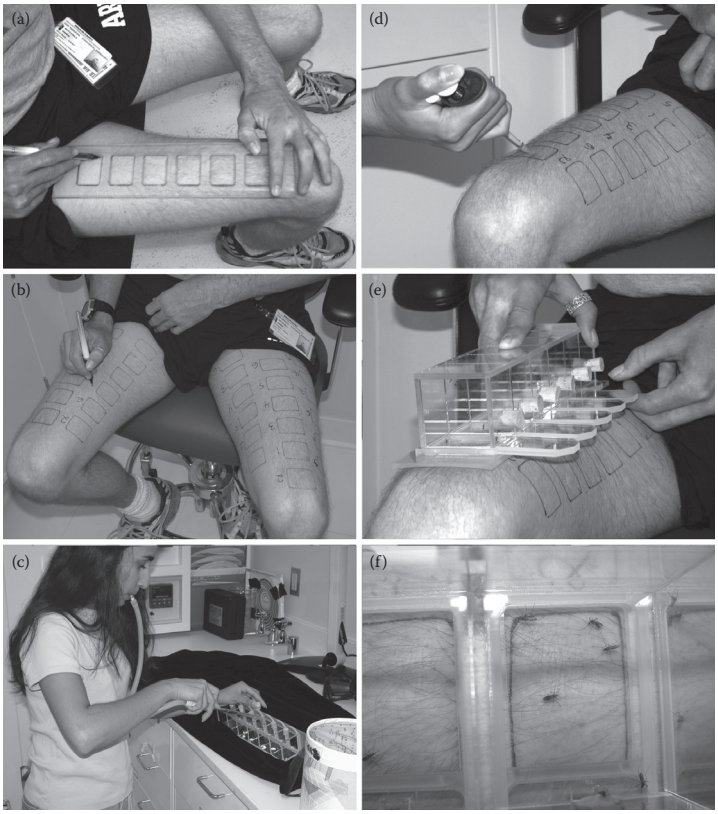

Figure 14.3 **(See color insert.)** (a) The seated volunteer uses an ink pen and an acrylic plastic template, representing the base and 3 cm × 4 cm openings of the K & D module, to mark skin areas of his thigh to be treated with 0, 3, 6, 12, 24, and 48 nmol/cm^2 skin doses of repellent chemical against *Aedes aegypti*. (b) Shows skin areas marked for treatment. Each row of six (3 cm × 4 cm) rectangular marks running down the volunteer's leg represents where a six-celled K & D module will be positioned on the volunteer's legs. Each row represents one replicate test of six repellent doses. Six treatments for each skin area in a row are randomly assigned for application to both legs of a person and yield six randomized replicates (blocks) per volunteer (in effect, a split plot design). (c) Shows the procedure for loading each of a module's six cells with five female *Aedes aegypti* from a 1 gallon screened carton holding 5- to 15-day-old male and female mosquitoes. Mosquitoes were usually maintained with sugar-water moistened cotton balls, but were provided water only 24 hours and no water for another 24 hours before being used in a bioassay. This treatment optimized the propensity of mosquitoes to feed in the bioassay. Once a set of mosquitoes have been transferred to a module, they should be utilized in the bioassay within 45 minutes to assure maximum biting propensity. (d) Shows randomized and replicated dose treatments being applied in 55 µL ethanol to marked areas of inner, top, and outer thigh skin surfaces. In applying a treatment, the solution is applied as uniformly as possible over the 3 cm × 4 cm and about 0.5 cm outside of the rectangular marking to assure that all skin surface subsequently exposed to the insects contains test chemical. Thus, the treatment solution is applied over a 4 cm × 5 cm area (20 cm^2) of skin, but the test insect is exposed only to a 3 cm × 4 cm area of skin. As a rule for general screening tests, chemicals being tested on human skin should be applied at a rate of 24–50 nmol/cm^2 skin. In this dose range, deet suppresses mosquito biting by about 80% compared to untreated skin.[11] (e) Sliding doors of each cell of the module are opened to expose the five mosquitoes in each cell to skin below for 2 minutes. (f) Mosquitoes are shown feeding on a control area of untreated skin after a 2-minute exposure to the skin. The number of mosquitoes probing the skin surface and engorging in each cell of the K & D module is recorded. Inspection of the figure shows that four of five mosquitoes are on the skin probing and engorging. The fifth mosquito is sitting on the plastic of the cell interior. The number of insects biting (in this case, four) in a cell is recorded and then its door is then slowly closed causing the mosquitoes to leave the skin surface and fly up into the closing cell.

IN VITRO K & D MODULE BIOASSAY SYSTEM

Despite the demonstrated effectiveness of the in vivo K & D bioassay in repellent research, limitation of its use to compounds known to be toxicologically safe constituted a severe restriction to chemical screening programs and discovery of new and effective repellents. This restriction provided impetus for development of an in vitro K & D bioassay system.[14] Our objective was to design a new bioassay system that would be equivalent to conducting assays using humans, but without the use of volunteers. Composition and organization of the system is shown diagrammatically in Figure 14.4. Figure 14.5 shows a picture of the system components.

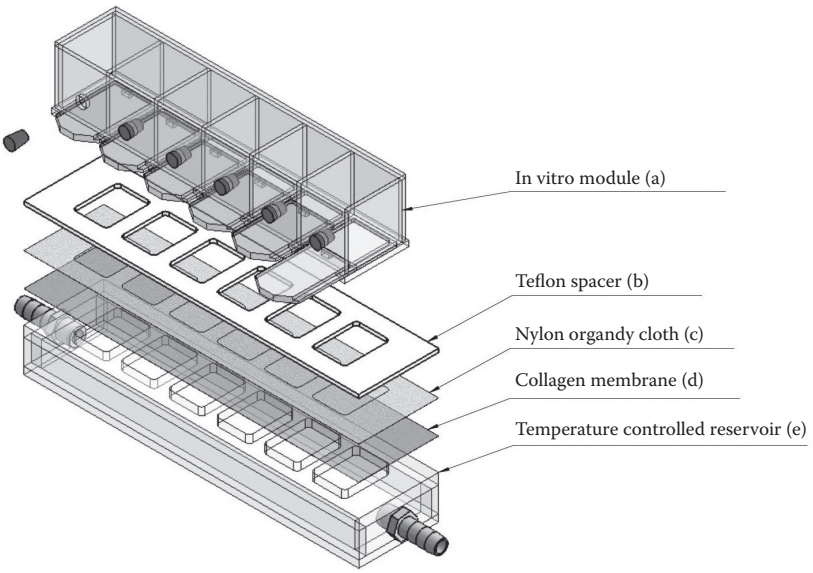

Figure 14.4 The in vitro module (a) for mosquito containment shown at the top of the diagram is identical to the in vivo module used with human volunteers (Figure 14.1) except the base of the module is flat, and not curved as was the case for the in vivo module. Component (b) is a spacer made of Teflon® that is identical to the base of the in vitro module having rectangular holes similar to the floor openings of (a). The purpose of the Teflon spacer is to prevent the module from contacting and becoming contaminated by test chemicals that are applied to organdy cloth (c). The cloth is marked with rectangular pen markings that complement the door openings of (a). Test chemicals in ethanol solution are applied to the marked rectangular areas on the cloth. The treated cloth is placed over an Edicol collagen membrane (d) that covers the six rectangular wells of a temperature-controlled (38°C) reservoir (e). The wells are filled with an aqueous preparation for mosquitoes to feed on and engorge. By design, the combined components (d) and (e) of the system represent a pseudo-human host for mosquitoes. They provide "pseudo-skin" to bite through, warmth (38°C), and a liquid below the membrane (pseudo-blood) to engorge upon. In our early use of the in vitro system, reservoir cells were filled with outdated human red blood cells suspended in anticoagulant-preservative (CPDA-1) obtained from a local blood bank supplemented with adenosine triphosphate (ATP) to cause biting mosquitoes to engorge.[15] Use of the blood cells raised significant health and logistic issues for conducting the bioassays. In 2008, we determined that citrate, phosphate, dextrose, and adenine (CPDA-1) and ATP (10^{-3} M) alone would stimulate mosquitoes to engorge.[16] This discovery led to elimination of human red blood cells from the bioassay, and enhanced the efficiency and biological safety of the assay. CPDA-1 aqueous solution, used as a mosquito-ingestion stimulus, was prepared by dissolving 3.33 g sodium citrate, 0.376 g citric acid, 0.28 g monobasic sodium phosphate, 4.02 g dextrose, and 0.035 g adenine in 126 mL water. This corrects a mangled recipe for CPDA-1 published in 2008[16] (printed as: 33.3 g sodium citrate, 0.376 g monobasic sodium phosphate, 4.02 g dextrose, and 0.35 g adenine in 63 mL water). If one has a 126 mL solution of CPDA-1 as presented here, and wishes to prepare a 10^{-3} M ATP solution from it, one would add 69.44 mg ATP (MW 551.14) to the 126 mL CPDA-1 solution. A convenient online molarity calculator for any given volume of solvent is available at http://www.physiologyweb.com/calculators/molar_solution_concentration_calculator.html.

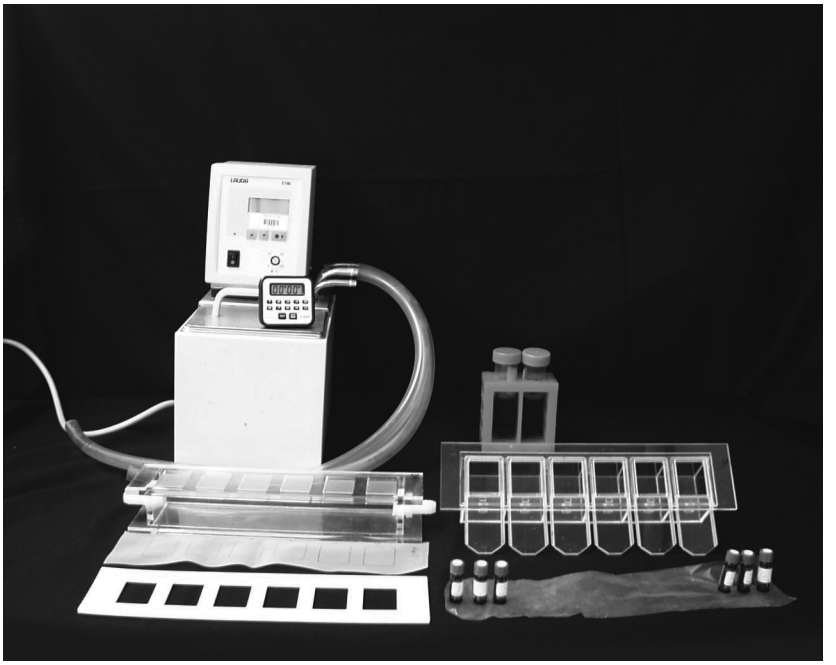

Figure 14.5 Picture of components used in the in vitro K & D bioassay. The picture shows a minute timer sitting on a water bath heater and cycling pump (38°C), and tygon tubing for connection to the six-well reservoir. Below the reservoir is a length of pen-marked nylon organdy cloth to which test chemicals will be applied. Below the cloth is the Teflon separator that was used as a template to mark the organdy cloth. A green rack holds containers of outdated packed red blood cells supplemented with adenosine triphosphate and it sits on an in vitro K & D module. Doors of the module are shown in an open position. In front of the module are six vials containing ethanol solutions of chemical treatments to be applied to the organdy cloth. The vials are standing on length of collagen membrane used to cover filled wells of the reservoir.

Figure 14.6 depicts the step-by-step use of the in vitro K & D bioassay system in a dose × response test of the repellents SS220, deet, and Picaridin. The response of *Aedes aegypti* to the three compounds was tested at 0, 3, 6, 12, 24, and 48 nmol/cm^2 cloth. The test was conducted using the same compounds and doses as was done earlier using the in vivo K & D with humans[11] (Figure 14.3). Results of the in vitro and in vivo tests with the four repellents are shown in Figure 14.7. The overall pattern of in vivo and in vitro results shows a similar decrease in biting (increase in nonbiting mosquitoes) with increased dose for all compounds. These comparative data are unique for the field of insect-repellent science in as much as we know of no other case where such a comparison of in vivo and in vitro test results has been published. As we found some differences between the in vivo and in vitro results (better compound separation and a different efficacy ranking in the in vivo system), final conclusions about a compound's utility are best drawn using the in vivo assay system after the compounds are toxicologically cleared for application to humans.

The in vitro K & D module bioassay with mosquitoes has proven to be a useful tool for discovery and characterization of chemicals that are effective against biting flies and ticks,[17–23] and a number of compounds discovered by using this bioassay have been patented.[24]

One theoretical issue that has not been fully resolved is that some attractant compounds might also inhibit feeding, especially at higher concentrations, which could also produce dose–response curves similar to those of repellents, and may be responsible for differences seen in olfactometer (where mosquitoes have the space to fly to or to avoid the test chemicals) and module testing (where mosquitoes are in small sealed cells). For example, lemon peel was found to be repellent in module

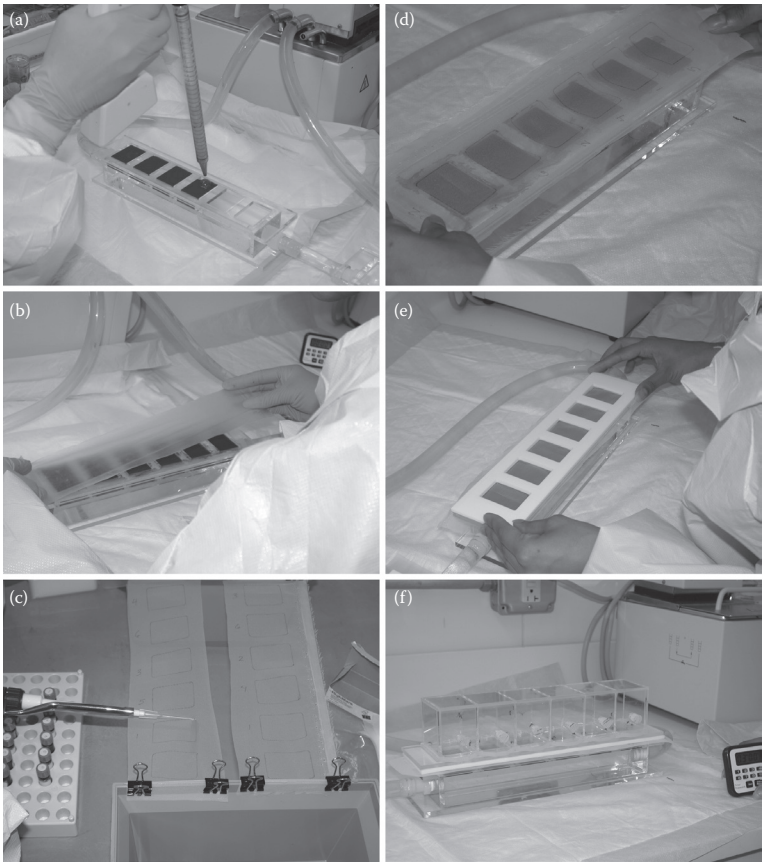

Figure 14.6 (a) Reservoir wells warmed (38°C) by water pumped from a water bath through the reservoir are filled with blood cells suspended in an aqueous solution of citrate, phosphate, dextrose, and adenine (CPDA-1) and adenosine triphosphate (ATP). Blood cells are pictured being used in this figure; however, a bioassay method developed subsequently[16] that uses an aqueous solution of CPDA-1 plus ATP alone, making use of blood cells in the bioassay obsolete. The recipe for CPDA-1 plus ATP is presented in the legend of Figure 14.4. Procedures shown in Figure 14.6 using blood cells would be the same if CPDA plus ATP are used. (b) A thin film of silicone grease is applied to edges of the reservoir and Edicol Collagen film (http://www.devro.com/our-products/edicol/edicol-a/) is secured over filled wells. (c) Five doses of compound at 3, 6, 12, 24, and 48 nmol/cm^2 cloth and ethanol alone (control) are applied uniformly to randomly marked cloth areas with a pipette. Cloth, suspended horizontally using paper clips between two trays, is treated 0.5 cm outside the 3 cm × 4 cm pen-marked area resulting in circa 20 cm^2 of treated surface. In screening tests of chemicals with unknown toxicology, treatments to cloth and the in vitro bioassays should always be made in a chemical fume hood such as a PURAIR ductless chemical fume hood (Air Science USA LLC, Fort Myers, FL). (d) Treated cloth is positioned over the Edicol collagen membrane. (e) Teflon separator is placed over the treated cloth. (f) K & D module with each cell containing five mosquitoes per cell is positioned over the Teflon separator. Mosquitoes are ready for exposure to chemical treatments. Doors of the module are opened to expose mosquitoes to treatments on the cloth surfaces, and the number of biting on the cloth surface of each treatment at the end of a 3-minute exposure is recorded. A 2-minute exposure period was routinely used in studies with in vivo human tests. The longer exposure time can be used with in vitro tests because there is no human discomfort. For high throughput screening, two reservoirs can be attached in series to a water bath with 38°C water pumped through both units. Using units in tandem increases bioassay capacity and efficiency. Empirical testing has shown that when two in vitro units are attached in series to a water bath pump, it is feasible for two technicians, working together, to screen at least 100 candidate-repellent compounds per 5-day week with 12 replicates/compound. (From Klun, J.E., Kramer, M., and Debboun, M., *J. Amer. Mosq. Control Assoc*., 21, 64–70, 2005.)

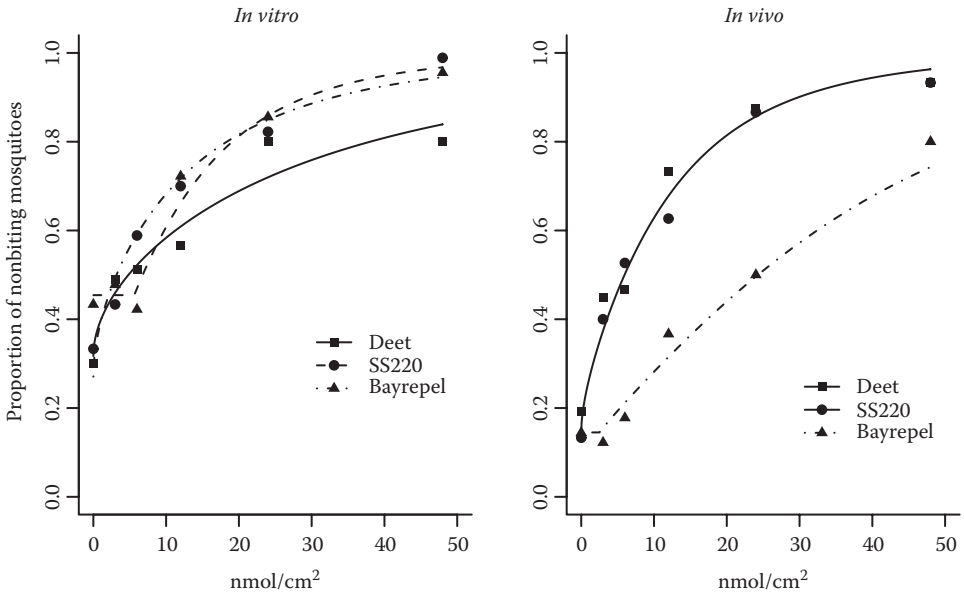

Figure 14.7 In vitro and in vivo dose–response relationships for deet, SS220, and Bayrepel for *Aedes aegypti* based on a generalized linear mixed model for the logit of the proportion of nonbiting mosquitoes. Empirical proportions of nonbiting mosquitoes calculated from the same data are also plotted with the estimated curves. Left panel: in vitro results. The dose–response lines for the three compounds are statistically indistinguishable, but best fit individual compound lines are depicted. Right panel: in vivo results. The model for deet and SS220 is logit (p) = $b_0 + b_C \sqrt{(\text{dose}_i)} + u_j$, that for Bayrepel is logit (p) = $b_0 + u_j$ for $\sqrt{(\text{dose}_i)} < 0.5$, and logit ($p$) = $b_0 + b_C (\sqrt{[\text{dose}_i]} - 0.5) + u_j$, otherwise; where p is the proportion of non-biting mosquitoes, i indexes the different doses, b_0 and b_C, are estimated parameters, where C indexes the three compounds, and u_j is the random effect of the jth subject, assumed to be a draw from a normal distribution with mean zero and variance estimated from the model fitting procedure (from Klun et al.[11]). The dose–response relationship for Bayrepel differs significantly from the other two.

testing but not in an olfactometer.[25] This is why we previously stated that K & D modules should be used only with chemicals having vapor pressures that are generally equivalent to that of deet. No system for testing mosquitoes has been shown to be definitive, in the sense of exactly mimicking what is found in field tests, which themselves are quite variable. As an example of the variability one finds in field tests, Traub and Elisberg,[26] in their Table 2, provide statistical summaries, where mean attack rates and their standard deviations are approximately the same for deet. Since mean attack rates have a hard lower limit of zero, this suggests that these data are strongly right skewed (highly variable). For the less protective combined insect repellent, M-2020 (also in their Table 2), mean attack rates and their variances are approximately the same (suggesting something similar to a Poisson process as a reasonable underlying model), again indicating high variability. However, the K & D module system is efficient and results are in basic agreement with other testing methods including field tests.

USE OF MODULES SIMILAR TO THE IN VITRO K & D MODULE BY OTHER RESEARCHERS

Tests of mosquitoes against various compounds of interest using a module system have also been conducted by Weldon et al.,[27,28] with a slightly different methodology, though using modules similar to those described above (the base had circular rather than square openings; the membranes used

were laboratory-made silicone wafers). The modules were placed over feeding wells, as described for the in vitro method above. Tests were run for 5 minutes. At the end of each minute, the behaviors of all (either five or six) mosquitoes in each cell in the module were recorded. Mosquitoes were categorized (three mutually exclusive categories) as either resting on the membrane (potentially feeding), flying, or resting on one of the other five surfaces of the cube. At the conclusion of the test, the module was closed and placed into a freezer to kill the mosquitoes, from which they were subsequently removed and squashed on a paper towel to determine if they had fed. If the feeding solution was clear (e.g., a sugar solution), green dye was added to it to make obvious those mosquitoes that fed. Additional details are given in the original articles cited earlier.[27,28]

The K & D module system was modified in a different way by Zhu[29,30] for use with stable flies, *Stomoxys calcitrans* (Linnaeus) and house flies, *Musca domestica* (Linnaeus). Three to five flies were placed in each cell of the module. Rather than using wells containing liquid, squares cut from a feminine hygiene pad were soaked either in citrated bovine blood for stable flies or in a red-dyed sugar solution for house flies, topically coated with the compound of interest, and were placed in the module wells. Tests were run for 4 hours. The dependent variables measured were whether individual flies fed (assayed by squashing the abdomens of the flies), time to knock down (flies lying on the floor of the box, unable to fly and abdomen up), and time to death (flies considered dead did not move when prodded with a thin wooden stick).

STATISTICAL ANALYSIS OF DATA FROM THE K & D AND SIMILAR MODULES

There are several useful kinds of data one can collect using this module system when testing compounds. The usual measure is whether or not a mosquito has ingested the feeding solution through the membrane. This can be determined visually (mosquito has mouthparts inserted through the membrane and the abdomen is distended and red), or the mosquitoes can be squashed at the test's conclusion. Blood feeding is easy to see, but food coloring dye should be added to colorless feeding solutions. Other behavioral measures can be useful, for example, one can census the mosquitoes in each cell at a given time point (or at several time points), and note the behavior of each mosquito (typically categorized as resting on the membrane, flying, or resting on one of the other five surfaces of the cell).[26,27]

Comparing Compounds and Concentrations Using Feeding

We first discuss the analysis of feeding counts, subsequently the analyses of other behaviors, how all behaviors observed can be combined into a composite score, and random effects useful in statistical modeling of these kinds of data.

To statistically model whether mosquitoes have fed, visually or using the squashed mosquito assay, we assume the mosquitoes are samples from a binomial distribution. That is, individual mosquitoes fed or not fed, and these counts are summed over the cell or cells. The binomial parameter, which models the proportion of feeding mosquitoes, depends in large part on how the chemical applied to the membrane affects feeding. If no chemical is applied (the control condition), most mosquitoes should feed. As chemicals become more effective, or are applied at higher concentrations, fewer mosquitoes should feed. There are other potential variables affecting the proportion of feeding mosquitoes, these include time of day, room environment (temperature, relative humidity), and mosquito characteristics (e.g., age, species).

The binomial distribution, unlike the normal distribution, has only one parameter, which, in conjunction with the known sample size (sample size is a constant, it is not estimated), determines both the mean and variance. The normal distribution has two independent parameters, one for the mean and one for the variance. The traditional way to handle binomial data was to use a variance

stabilizing formula, for example, $\sin^{-1}\sqrt{y/n}$, where y is the number of feeding mosquitoes (an equivalent analysis defines y as the proportion of nonfeeding mosquitoes) and n is the total number of mosquitoes. An improved formula is $\sin^{-1}\sqrt{\left(y+3/8\right)/\left(n+3/4\right)}$. One then applies statistical tests for normally distributed data (e.g., *t*-test, ANOVA). With modern statistical software, use of these transformations is no longer necessary. In fact, the transformations do not work well when y/n is small, as occurs when the chemicals tested are effective, nor when y/n is near 1.0, for example, for controls. The current preferred method is to use software that estimates a generalized linear model[31] using the logit link for binomial data, sometimes called logistic regression. Some prefer the probit to the logit link, the differences between them are small. Essentially, instead of modeling the proportion directly, one models the expected value of the logit of the proportion, $p = y/n$, that is, E [log $(p/\{1 - p\})$], where the logit (p) (the dependent variable) is influenced by chemical, concentration, environment, and mosquito characteristics (the independent variables), and E is the expectation function. These models have better statistical properties for modeling binomial data than using transformations. Statistical tests for differences between chemicals will be more accurate, other independent variables can easily be added to the models in a regression-like manner to look at their influence, the change in the variance, as a function of the mean, is correctly accounted for, and confidence intervals will make sense. When using a transformation, it is quite possible to have a 95% confidence interval on a proportion that includes negative numbers.

However, analyzing data in this framework reveals a weakness of basing a statistical model on the binomial distribution because biological data does not usually conform to theory. In particular, for binomial data, there is usually more variability than expected from a pure binomial process, even taking into consideration the other independent variables mentioned earlier. This unaccounted for additional variability is known as over-dispersion. The fix is to add an over-dispersion parameter to the model, which corrects the often too small standard errors and make appropriate adjustments on statistical tests. An alternative fix is to include random effects at the individual level, for example, for each cell, although not all software will allow this. Another problem occurs if there are compounds that were 100% repellent, where no mosquitoes fed at that compound/concentration combination. Including these data in the analysis without adjustment leads to problems, typically by producing enormous standard errors in the output (when actually, at a proportion of 0% or 100%, the variance is zero). There are two remedies: either do not include the data in the analysis, which makes sense because if the variance is zero, there will be a significant difference between this compound and any other, or change one of the zero bites to a fraction (0.1); even though binomial data consists of counts, putting in a fraction rather than an integer does not break the software, though it may produce a warning message. However, any comparisons made involving this compound are suspect because the data have been altered.

In the framework of a generalized linear model, linear model decompositions (contrasts) are done in the usual traditional way. For example, if one compares controls against each of the test compounds, this is an a priori linear contrast and sufficient degrees of freedom exist to avoid a multiple comparisons adjustment. However, if one also compares each compound to every other one, or against a deet positive control, then one is making more comparisons than allowed for with the degrees of freedom (the multiple comparisons scenario) and an adjustment, either on the test statistic or the p value, is needed. A Bonferroni adjustment is an example of an adjustment on the p value; better methods exist—a contemporary one is to adjust for the false discovery rate.

Often a compound is tested at several concentrations with the aim of constructing a "dose–response" curve. We suggest use of the generalized linear model framework. The key is to find an appropriate transformation of the concentration so that the relationship between the logit (or probit) of the proportion of bites and concentration is a straight line. If there is only one compound involved, this is usually not difficult, but sometimes a transformation on dose creates a straight line relationship for one compound but not for others, which is problematic because the goal is to

compare linear slopes. In that case, the slopes can only be compared in regions of concentration where the same transformation can be used for all compounds. An alternative is to fit a polynomial (linear and quadratic components) to the transformed concentration, and compare and interpret both components. In our work, we have transformed concentration using the log, square root, and identity (no transformation).

Some compounds seem to be completely ineffective at low doses, but then exhibit a normal dose–response curve at higher concentrations. In this case, we have modeled the dose–response curve as flat, that is, no change from control, until some low subjectively estimated concentration, then rising from that point (Picaridin in Figure 14.7). In the statistical model, one simply subtracts this low estimated concentration from all the others so it becomes the new "zero" and then estimates the model in the usual way. For high concentrations, where no mosquitoes feed, the same reasoning applies as discussed earlier, either those concentrations should be dropped from the analysis or the data need to be slightly altered to allow the curve to be fit. However, in this case, one can choose how to alter the data such that this final high concentration does not affect the model fit (the altered point sits close to the line fitted without the point). Neither of these alternatives for fixing compound/concentration combinations of zero is satisfying. In theory, the zero proportion estimate results from sampling error. That is, if sufficient numbers of mosquitoes were tested, at least one would have fed, so the true value at this concentration is nonzero.

Including controls, where no compound is applied to the membrane, provides both a measure of experiment-to-experiment consistency and a baseline against which to measure the activity of compounds of interest. Kramer et al.[32] investigated the statistical properties of testing mosquitoes in modules and found that the correlation between control biting rates and treatment biting rates on deet-exposed membranes in the same trial was 0.67 (p = .002). It is unclear how to interpret this number. Although there is clearly a strong relationship between the two, it is not strong enough for the control values to be used to adjust the deet values of different experiments to a common level. There were even trials where bites by mosquitoes in the deet treatment approached or exceeded those by controls. Further, they found that, ignoring sampling error, most (61%) of the variation among controls was not accounted for (i.e., was not due to day-to-day or session-to-session variability), indicating that analyses for these kinds of data need to allow for over-dispersion, discussed earlier. These results also suggest that large sample sizes be used, because that will allow for a good estimate of the additional unaccounted for uncertainties as an over-dispersion parameter. Luckily, for mosquito researchers, large samples sizes are easy to obtain.

Comparing Compounds When Additional Behaviors Have Been Recorded

The proportion of landing or flying is analyzed in a different way if mosquitoes are repeatedly measured, that is, every minute in a 5-minute test, as done by Weldon et al.[27] Mosquitoes at each observation period were classified into one of three categories, landing on the membrane, landing elsewhere, and flying, for example, Weldon et al.,[27,28] but only two of the three categories were analyzed because the sum of the counts in all categories is always the product of the number of mosquitoes in the cell and the number of times measurements were made, and the tests are then not independent. Because one is repeatedly measuring the same unmarked individuals and then summing all the counts, the data are no longer strictly binomial, which assumes the counts are independent. Given that, in a 5-minute test, one could have a maximum count of 25, one can apply the standard (or improved, given earlier) variance stabilizing transformation for proportions, on count/25. The transformed proportions can then be analyzed using a statistical model for normally distributed data, for example, ANOVA. This was the approach taken by Weldon et al.[27,28]

If there are two or more concurrently measured behaviors on each group of five mosquitoes, for example, landing on the membrane, flying, and feeding, an alternative that we have found works well

to rank a large number of compounds tested concurrently is to use the methods given in Kramer et al.,[33] where a composite score is created (a single score for each cell of five mosquitoes based on optimal weightings of the concurrently measured behaviors) that maximizes the differences among compounds. This method was used for mosquitoes in Weldon et al.[25] If other variables, for example, time of day, had a large influence on the outcome of tests, those variables could also be included when creating the composite score. If the resulting scores are close to normally distributed, then the usual linear models can be used to test for differences. In Weldon et al.,[25] where distributions of the resulting scores were decidedly skewed, differences were tested using the a posteriori Kruskal–Wallis method.[34]

RANDOM EFFECTS

In general, any time humans or other vertebrates are used as potential hosts in an experiment, there are individual differences in their attractiveness to mosquitoes, and this source of variation should be included in the statistical model. These are random effects because, if the experiment was run again, likely a different set of potential hosts would be used (versus a fixed effect like concentration or time of day). Another potential random effect that may need to be included in the statistical model is a block effect, for example, if the experiment was repeated over a few days, there may be a random day effect. If random effects are included in the model, then the model framework changes to linear mixed models or generalized linear mixed models. Current statistical software can estimate these models and they should be used because they more accurately reflect the process producing the data and thus give better statistical tests. However, since the software is relatively new, it is also less mature, and estimation problems are more likely, especially if there are compound/concentration combinations with no mosquitoes feeding, as mentioned earlier. Also, model diagnostics are not as far along, though the situation should improve with time.

CONCLUSIONS

K & D Module Use

The K & D module system was developed and overcame problems in previously used module systems. By creating cells containing only a few mosquitoes, a larger number of compounds can be concurrently tested, which is both a better statistical design that allows for higher throughput when screening, and uses fewer mosquitoes. Even higher throughput can be achieved by eliminating human volunteers and testing mosquitoes in an in vitro system, where they can feed through a membrane under which lies wells of a blood substitute, kept warm using circulating water. This methodology has been adopted by other researchers, demonstrating the usefulness of the system.

Data Analysis

If only one dependent variable (feeding) is observed, data analysis is straightforward, although over-dispersion of the data (relative to a binomial distribution) is typically present and needs to be taken into account in the analysis. If more than one dependent variable is observed, the composite score technique[33] is effective for reducing the dimensionality of the data. Random effects are also typical of the experimental designs used, such as those resulting from different volunteers in in vivo trials, day-to-day differences, and so on and should not be ignored because doing so makes for too liberal tests (p values are too small). They can be included if the data are analyzed in the generalized linear mixed model framework.

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CHAPTER 15

Use of Chemical Mixtures as Insecticides and Repellents

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CONTENTS

INTRODUCTION

Research on Insecticidal and Repellent Mixtures

Research on this topic requires careful consideration of the chemical composition as well as the response endpoint. Chemical mixtures might constitute any number of components, but not all components contribute directly to the biological activity. Some components may function as true inerts and their presence/absence does not affect overall insecticidal or repellent activity. The impact of each individual chemical can be quantified, but it requires careful consideration of the method of application and the number of target sites that are involved in the biological response. It can be particularly challenging in complex mixtures involving multiple active components to quantify the intensity of the interaction in a biological system when there are mixed exposures and multiple target sites.[1] In particular, assessment of chemical dose–response relationships can be further expanded to include constraints on mathematical models. For the sake of simplicity, examination of the interaction potential for binary mixtures is discussed in this chapter. These approaches to the analysis of mixtures are increasingly common in the pharmacological and toxicological sciences. There is a need for standardized and simplified practices to ensure the quantification of valuable and meaningful interaction parameters for research.

Classical approaches to quantifying interactions can provide an outline for the different scenarios under which chemicals will interact in combination with each other.[2,3] Key assumptions pertaining to independence are incorporated into these models and they should be considered before selecting a set of definitions. However, the conceptual approach to interactions including synergism, additivity, and antagonism is outlined in Table 15.1.[4] The Loewe model addresses additivity and takes the position that an agent does not interact with itself. Therefore, one would expect that the combined effect of the agent (either by increasing dosage or by adding another similar acting agent) would result in an additive effect. Bliss independence states that each agent acts independently of the other agents that are present. Other combination effects might include cases where only one agent or neither agent is predicted to result in a response including cases of inertism and coalism.

There are numerous ways to approach studies on chemical mixtures, and part of the challenge that remains in insecticide and repellent research is selecting the appropriate model for the analysis of the combined effects; especially when information on the mode(s) of action are sometimes limiting. The more simplified and qualitative approach of Berenbaum[5] and Laska[6] offers options including variations of Loewe additivity and tests for significance (Figure 15.1).

Future work on insecticidal and repellent mixtures would largely benefit from the use of a standardized approach to analyzing interactions within insecticidal and repellent chemical mixtures. There are simplified methods developed to test for statistical significance of combination

Table 15.1 Loewe and Bliss Models for the Combination of Effects Resulting from Two Agents, as Compared to the Reference Model

	Agents Effective Individually (Loewe Model)	Agents Effective Individually (Bliss Model)	One Agent Effective	Neither Agent Effective
Response greater than predicted	Loewe synergism	Bliss synergism	Synergism	Coalism
Response equal to prediction	Loewe additivity	Bliss independence	Inertism	Inertism
Response less than predicted	Loewe antagonism	Bliss antagonism	Antagonism	

Source: Greco, W. R. et al., *Pharmacol. Rev.*, 47, 331, 1995.

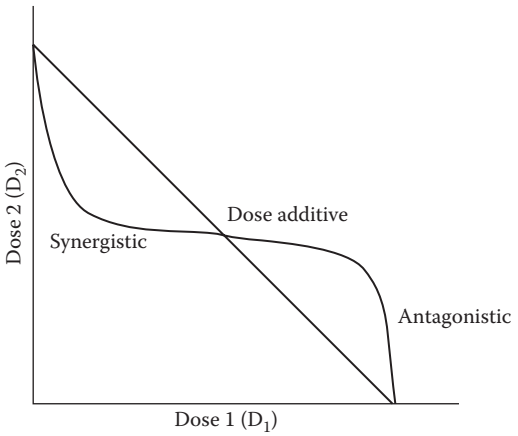


Figure 15.1 Antagonistic and synergistic representation of agent combinations (D_1 and D_2) and dose–additive line. (Adapted from Laska, E. M., Meisner, M., and Siegel, C., *Biometrics*, 50, 834–841, 1994.)

effects, and more robust studies could focus on generating response curves based on quantitative dose–response relationships.[4]

Success of a Pesticide Mixture: Pyrethrum

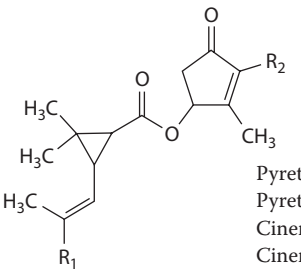
A review of the research into the composition of pyrethrum and the resulting development of it into one of the most widely used insecticide classes, pyrethroids, reveals many of the limitations and complexities associated with insecticidal and repellent mixtures. Pyrethrum, first utilized in the form of ground-up flower heads of *Chrysanthemum* spp., was used to control flea and lice around the 1800s.[7] Later, it was determined that pyrethrum was a mixture of four active toxic esters: pyrethrins I and II, and cinerins I and II. Research on these esters led to the development of the synthetic analogs, the first of which was allethrin, made in 1949 by La Forge and associates.[7] Initially, there was a market for allethrin in the use of mosquito coils, but on a cost basis, pyrethrins were still more efficient.[8] Further work on structural deviations from the naturally occurring esters found in *Chrysanthemum* led to the replacement of pyrethrum formulations in the 1970s. Mainly, this was due to the research of Dr. Michael Elliott and Dr. Norman Janes at the National Research and Development Corporation in England, where they focused on synthesizing new pyrethroids (resmethrin, bioresmethrin, permethrin, cypermethrin, and deltamethrin). Their efforts were brought to the attention of the major agricultural chemical companies and, in time, licenses were secured. This led to the testing, registration, and commercialization of the class of insecticides known as pyrethroids.[9] Once the commercial utility of the pyrethroids had been established, even more research was directed to structure modifications. Japanese researchers, including Dr. Junshi Miyamoto at Sumitomo, made structural improvements that increased insecticidal activity, photostability, and decreased mammalian toxicity. As these improvements were introduced into the marketplace, their order in the sequence of the discovery process was denoted by classifying the product as first, second, third, and fourth generations of pyrethroids. In the United States, as of 2012, there are over 3500 Environmental Protection Agency–registered formulations containing pyrethroids.[10] Thus, these chemistries have had a significant impact on insecticides available for use around the globe, but none of this would have been possible if researchers had not been able to ascertain the active ingredient content and potency of the natural pyrethrum plant extract. Without the ability to elucidate the various esters of pyrethrum and to determine the toxicity of mixtures, this entire class of insecticides, pyrethroids, would have not been developed.

Early Pyrethrum Work: Active Ingredient Identification and Formulation

In early research, the dried and powdered flower heads of *Chrysanthemum cinerariaefolium* were referred to as pyrethrum or insect powder. Pyrethrum extract, on the other hand, referenced a solvent extraction of the flower heads. Some of the earlier pyrethrum sprays included mixtures of petroleum oils, coal-tar creosote oil, naphthalene, pine oil, pine tar oil, and *para*-dichlorobenzene.[11] Common constituent groups of the pyrethrum extract were described in the Pyrethrum Blend FEK-99 (Shrader Laboratories, Detroit, MI), including pyrethrins (68.2%), hydrocarbons (15.1%), terpenes (8.5%), high molecular weight hydrocarbons (7%), and other inerts (1.2%)[8] (Figure 15.2).

Studies on Chemical Composition and Biological Activity

Pyrethroids negatively impact insect systems by binding to the Na+ channel and resulting in rapid depolarization of the nerve axon. This class is well known for characteristic knockdown effects on flying insects, and with sublethal exposures, and excitorepellency.



Pyrethrin I: R1 = CH_3; R2 = $CH_2CH=CHCH=CH_2$
Pyrethrin II: R1 = CO_2CH_3; R2 = $CH_2CH=CHCH=CH_2$
Cinerin I: R1 = CH_3; R2 = $CH_2CH=CH_3$
Cinerin II: R1 = CO_2CH_3; R2 = $CH_2CH=CH_3$
Jasmolin I: R1 = CH_3; R2 = $CH_2CH=CHCH_3$
Jasmolin II: R1 = CO_2CH_3; R2 = $CH_2CH=CHCH_3$

Figure 15.2 Pyrethrum extract.

Insect Repellent Mixtures and Combinations

One of the earliest insect repellents developed from a mixture or combination of chemical compounds was known as Sta-Way Insect Repellent Lotion.[12] This mixture repellent was developed at Rutgers University after workers tested about 1000 organic chemicals and chemical mixtures. It was composed of diethylene glycol monoethyl ester, ethyl alcohol, corn oil, and perfume. The idea of using mixtures and combinations of insect repellents was developed to get a broader range of efficacy,[13] which resulted in the development of repellent products such as dimethyl phthalate (DMP), ethyl hexanediol (EH), indalone, and dimethyl carbate (DMC). This led to the development of the combined insect repellent product known as 6-2-2 or M-250 that consisted of six parts of DMP, two parts of indalone, and two parts of EH, which became the standard U.S. military insect repellent in the latter part of World War II. M-250 as a topical insect repellent provided good protection for 4–6 hours[14] from *Aedes flavescens*, yellow fever mosquito, *Aedes aegypti* (L.),[15] and from the tsetse fly, *Glossina morsitans*. The next insect repellent combination, adopted as the U.S. military standard topical insect repellent in 1951, was known as M-2020 and consisted of four parts DMP, three parts EH, and three parts DMC.[16] Also, in 1951, a new insect repellent mixture, M-1960, was adopted as the standard clothing insect repellent for the U.S. military.[17] M-1960 consisted of 30% 2-butyl-2-ethyl-1,3-propanediol for protection against mosquitoes and other biting flies, 30% *N*-butylacetamide for ticks, 30% benzyl benzoate for chigger mites and fleas, and the remainder being Tween 80 as an emulsifier. This repellent was applied to clothing during the World War II in the Pacific Theater and was successful in disrupting and stopping the devastating effects of scrub (chigger-borne typhus). M-2020 was also recommended to be used in conjunction with M-1960 against mite bites.[18] Later, M-2020 was found to provide good protection against the malaria mosquito, *Anopheles albimanus*, for 3.1 hours,[19] whereas another mixture of 20% deet and 15% EH provided 12 hours of protection against *Anopheles* mosquito bites in Senegal.[20]

In a laboratory study to evaluate the feasibility of combining two or more insect repellent compounds for repellency against a broad range of medically important arthropods, Debboun et al.[21] found out that the repellent combinations of 2-hydroxy-methyl-cyclohexyl acetic acid lactone (CIC-4)/1-[3-cyclohexen-1-ylcarbonyl]-2 methyl piperidine (AI3-37220)/1-[3-cyclohexen-1-ylcarbonyl] piperidine (AI3-35765), deet/AI3-35765, and deet/AI3-37220/AI3-35765 against *Anopheles stephensi* and CIC-4/AI3-35765, deet/AI3-37220/AI3-35765, AI3-37220/AI3-35765, and CIC-4/AI3-37220 against *Aedes aegypti* were more effective than the component repellent compounds alone. This study provided, for the first time, quantitative and comparative data indicating some evidence of synergistic interaction between the repellent compounds.

In a field study in Burma with a sample size of 897 women conducted by McGready et al.,[22] Burmese pregnant women using a mixture of thanaka and deet experienced a 28% greater reduction

in the incidence of *falciparum* malaria than women using thanaka alone. In addition, the combination of thanaka and deet provided protection for over 10 hours against *Aedes aegypti*. Thus, the use of the combination of thanaka and deet was strongly recommended, and more women expressed a preference to the thanaka and repellent mixture than other insect repellents alone. Earlier, in another field study in Thai-Myanmar, Lindsay and coworkers[23] found that the repellent combination of 20% deet and 0.5% permethrin reduced exposure to malaria parasites by 65% and 85% against *Anopheles minimus* and *Anopheles maculatus*.

A repellent soap from Australia containing 20% deet and 0.5% permethrin was used successfully in Malaysia,[24] Papua New Guinea,[25] Australia,[26] India,[27] Ecuador and Peru,[28] and Pakistan.[29] In another field study in Guatemala and Peru, a low-cost repellent that was a mixture of *para*-menthane-diol and lemongrass oil provided 95% protection against *Anopheles* mosquitoes for 6 hours whereas 20% deet alone provided significantly lower protection of 64%.[30] Recently, due to the development of pyrethroid resistance in mosquitoes, the use of a combination of an insect repellent and insecticide-treated bed nets or mixtures of insect repellents and nonpyrethroid-treated fabrics has become new promising tools for disease vector control.[31–33] For example, the combination of the insect repellent deet and a nonpyrethroid insecticide propoxur provided a significantly higher mortality rate (96%) against the susceptible and pyrethroid-resistant strains of *Aedes aegypti* mosquitoes due to a strong synergism between deet and the propoxur.[31] Similar synergism was also observed in another laboratory study where the repellent mixtures of deet plus pyrimiphos methyl (PM), an organophosphate, and the repellent hydroxyethyl isobutyl piperidine carboxylate (also known as Picaridin or KBR 3023) plus PM provided 95% protection against the malaria mosquito, *Anopheles gambiae*, for more than 2 months compared to less than 1 week for each compound used alone, thus showing a strong synergy between the repellents and PM.[32] In another field study in Burkina Faso, Pennetier and coworkers[33] compared the efficacy of mosquito bed nets impregnated with mixtures of deet plus PM or KBR 3023 plus PM with mosquito bed nets treated with a standard formulation of deltamethrin and found out that the mixture of an organophosphate (PM) and an insect repellent (deet or KBR 3023) on bed nets was as effective as deltamethrin alone and more effective against the resistant *Anopheles gambiae* populations. Recently, Faulde and Nehring[34] found that the knockdown activity of the combination of deet/permethrin long-lasting insecticide and repellent-treated nets (LLIRNs) was significantly better than deet or permethrin concentrations alone. Similarly, the knockdown activity of the combination of deet/etofenprox LLIRNs was significantly improved than the deet concentration alone. Thus, these results demonstrate that the concept of mixing an insect repellent with an insecticide provides an alternative to the use of pyrethroids on bed nets against mosquito populations.

Essential Oils

Historically, Bacot and Talbot[35] were among the first to study various mixtures of essential oils. Bishop[36] recommended a mixture of citronella and pennyroyal, whereas MacNay[37] reported successful use of a mixture of concentrated oil extract of pyrethrum, oil of thyme, and castor oil against *Aedes* mosquitoes in Canada. Although many plant essential oils have shown insect repellency, their commercial application, to date, has been limited because their repellency is of a limited duration, mostly due to the volatility of their active ingredients.[38] To compensate for and improve these shortcoming factors, researchers have conducted studies to examine essential oil mixtures combined with fixatives and adjuvants such as kerosene, olive oil, tamanu oil, and vanillin,[39–43] the results of which showed better repellency than the individual essential oils. Results from studies with mixtures of cassia, lemongrass, lemon eucalyptus, and *zanthoxylum* oils with vanillin indicated they were promising effective mosquito repellent products.[44–46] For example, a mixture of lemongrass oil, xanthoxylum oil, and vanillin provided 270 minutes of complete protection time (CPT)

compared with 247.5 minutes of CPT with 15% deet against *Aedes aegypti*,[45] demonstrating that these plant essential oil mixtures combined with vanillin showed good repellency to *Aedes aegypti*. Khan et al.[46] showed that mixtures of deet and vanillin when compared to deet alone increased the repellency duration time against mosquitoes from 5 to 12–14 hours compared with deet alone.

The effectiveness and duration of repellency of essential oils also depend on their mixtures of hydrocarbons such as terpenes; oxygenated compounds such as esters, aldehydes, ketones, alcohols, phenols, and oxides[46,47]; and the frequency of the application and the formulation used.[48,49] In a study conducted by Hieu et al.,[50] they found that there was definitely an increase in repellency when using mixtures of seven essential oils (clove bud, clove leaf, thyme white, patchouli, and savory) and tamanu oil against *Stomoxys calcitrans* (L.) when compared with that of the constituted essential oil, deet, or tamanu oil alone. Kwon et al.[51] evaluated the repellency of *Aedes aegypti* to *Zanthoxylum armatum* seed oil (ZA-SO) alone or in combination with vanillin, its six constituents, and another *Z. piperitum* fruit oil constituents, as well as aerosol products containing 5% or 10% ZA-SO and 5% vanillin and compared the results with those of deet as a standard. The mixture of ZA-SO and vanillin provided a significant increase in repellency and duration of effectiveness over a 90-minute interval when compared to deet alone. Thus, these researchers concluded that binary mixtures of ZA-SO and vanillin could be useful as insect repellents for protecting humans and domestic animals from mosquito bites and nuisance. Other researchers have also reported that the repellency duration against mosquitoes was more pronounced in mixtures of a repellent essential oil and vanillin than that of a single essential oil.[40,52,53] Recently, Gallardo et al.,[54] evaluated the four major components of geranium oil and their mixtures for pediculicidal activity and synergy against *Pediculus humanus capitis* De Geer and found that the toxicity of the four mixtures was more toxic than the geranium oil showing a significant presence of synergistic interactions among the mixtures. Similarly, Jiang et al.[55] in a comparative toxicity study of essential oils of *Litsea pungens* and *L. cubeba* and blends of their components against cabbage looper, *Trichoplusia ni*, also indicated a synergistic effect among their constituents. Recently, in a study in the Republic of Korea, the effectiveness of a binary 1:2 mixture of thymol and vanillin was found to be significantly more effective against *Aedes albopictus* than thymol alone for a period of 2 hours.[56]

CONCLUSION

This chapter has shown that the use of mixtures of insecticides and repellent chemicals provide improved protection against biting arthropods, compared with individual chemicals alone. More than 70 years ago, researchers showed that mixtures of synthetic repellents provided longer and broader spectrum of protection than individual chemicals alone. In recent years, plant essential oil mixtures, synthetic repellents, and insecticides have shown enhanced protection against a wide range of biting arthropods. The use of chemical mixtures will remain an integral and important part of improving the limited tools available for protection against vectors of disease pathogens, their annoyance, and bites.

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CHAPTER 16

Use of Repellents Formulated in Specialized Pheromone and Lure Application Technology for Effective Insect Pest Management

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CONTENTS

INTRODUCTION

An insect repellent is a chemical compound or blend of compounds that deters insect activity on or near otherwise attractive substrates. Although repellents have played a key role in the

control of hematophagous insects and a large number of repellent semiochemicals, including some pheromones have already been characterized for agricultural and forestry insect pests, they are not widely commercialized in agriculture or forestry.[1–3] The vast majority of repellents are labile semiochemicals that quickly vanish once applied in the field, as conventional slow-release formulation technologies are often difficult to apply and/or inefficient in controlling the emission rate of the active ingredient. Although the use of repellents, alone or in combination with attractants as part of a push–pull strategy, has been shown to be effective in agriculture and forestry systems, the effective application of such compounds requires the user to have a more comprehensive knowledge of insect behavior than the use of conventional insecticides, which are not only simpler to use but typically less costly.[1,4]

The specificity of semiochemical repellents, which are often best adapted for a limited number of insect species in a restricted number of crop or forest systems, presents another challenge to their successful implementation on a large scale, as do the intricacies of the U.S. Environmental Protection Agency's (EPA's) registration process for new biopesticides, which can be very costly and time consuming.[1,2,5] This small-sized specific market, combined with the high cost of product development and registration, hampers the commercialization of repellent technologies, especially for minor crops or tree species. Despite the many impediments to commercialization of insect repellents, there are some situations where their use in agriculture and forestry is desirable and warranted. ISCA Technologies (Riverside, California), together with collaborators from academic, government, and private sectors, is actively developing novel repellent formulations against several important pest species. Here, we describe two case studies utilizing ISCA Technologies' controlled-release matrix SPLAT® (Specialized Pheromone & Lure Application Technology): SPLAT Verb, a repellent for the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, a pest of lodgepole, *Pinus contorta* Douglas ex Loud., and other pines; and SPLAT ACP Repel, a repellent for the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, a serious pest of citrus plants.

Insects inhabit a complex, constantly shifting, olfactory landscape, comprising a plethora of different volatiles emanating from the biotic and abiotic environment. Olfaction is often considered the prevalent sense that mediates insect behavioral sequences resulting in host selection.[6,7] Many insects possess sophisticated olfactory systems equipped with numerous olfactory receptor proteins[8] expressed on the dendritic membranes of sensory neurons housed in olfactory sensilla. The recognition of a host plant by insects is believed to be based on either specific olfactory receptors/olfactory sensory neurons that detect specific odorants released from a specific plant or combinations of olfactory receptors/olfactory sensory neurons that together detect specific ratios of general odorants in a blend.[9] Olfactory receptors represent the molecular basis for the specificity of olfactory sensory neurons.[10] Many different herbivorous insects have olfactory sensory neurons/olfactory receptors tuned to the components of commonly occurring green leaf volatile alcohols and aldehydes, which are major constituents of green plants.[11–13]

Phytophagous insects may have the ability to discriminate between hosts and nonhosts, as well as between hosts of different quality.[11,14] For example, herbivore-induced plant volatiles are important signals for an ovipositing female, allowing her to judge the quality of the host plant before laying eggs, which is a crucial decision for the survival and development of her offspring. Volatiles released from nonhost plants are also important cues that may warn insects to avoid nonhost or less preferred host plants, or enable them to select the right habitat and a suitable host plant. For example, nonhost volatiles may modulate host-selection behaviors of bark beetles by reducing their attraction to pheromones or host kairomones, or by enhancing the effect of antiaggregation pheromones.[3,15] The large number of attractive and nonattractive volatiles released by plants, combined with differing combinations, constitutes a major challenge for herbivorous insects attempting to identify their host plants in a complex olfactory landscape.

Specialized Pheromone and Lure Application Technology

Although most semiochemical controlled-release formulations have taken the form of devices, such as aerosol dispensers (Puffer®, Suterra, LLC), polyethylene tubes (Isomate®, Shin-Etsu Chemical), semipermeable plastic membranes (BeetleBlock™, Synergy Semiochemicals), and laminated polymers (Disrupt®, Hercon Environmental), ISCA Technologies has taken an alternative approach: the controlled-release emulsion SPLAT, which is a unique technology that can be adapted to dispense a wide variety of compounds, including semiochemicals, pesticides, and phagostimulants, while protecting them from degradation across a broad range of diverse environments. Although adapting SPLAT to release new compounds can pose major technical challenges, the versatility of this flowable formulation provides many compensatory benefits. SPLAT emulsions can be designed to hold a vast array of semiochemical concentrations and additives to create a formulation that releases the optimal rate of a given semiochemical for a desired period, while shielding active ingredients from environmental, chemical, and biological degradation. In addition, the rheological properties of SPLAT can be adjusted to create emulsions with a wide range of physical properties, allowing the use of a variety of manual and mechanized application techniques (Figure 16.1 and Figure 16.2). Unlike most other semiochemical dispensers, SPLAT is not restricted to a particular point source size. Any amount of SPLAT constitutes a point source, providing yet another way to optimize application rates and coverage in the field. Furthermore, the biodegradability and low manufacturing cost of SPLAT significantly decrease environmental impacts and enable commercialization of more affordable semiochemical-based control products.

Figure 16.1 **(See color insert.)** SPLAT Verb for protecting individual *Pinus contorta* from *Dendroctonus ponderosae* attack was applied with mechanical application equipment housed in the bed of a John Deere Gator (left, center). The same system can be adapted to a helicopter, airplane, tractor, or pickup truck. *Pinus contorta* baited with a *Dendroctonus ponderosae* tree bait (brown pouch) following application of SPLAT Verb during a pilot study (right).

Figure 16.2 Mechanical application of SPLAT. Here, SPLAT is dispensed from a 57-L drum using a pneumatic pump supplied by an air tank (alternatively, a gas-powered air compressor may be used). This basic model can be replicated in any number of ways depending on the equipment available and field characteristics.

MOUNTAIN PINE BEETLE, *DENDROCTONUS PONDEROSAE*

Dendroctonus ponderosae is native to forests of western North America and is one of a few species of bark beetles that behave as true predators in that host colonization typically results in mortality of the host tree.[16] The extent of tree mortality resulting from *Dendroctonus ponderosae* may be limited to small spatial scales (e.g., individual trees or small groups of trees at endemic population levels) or may affect entire landscapes. During the early stages of an outbreak, the beetles typically target trees already under stress from other sources,[17] such as mechanical injury, drought stress, other insects, root disease, and/or old age. *Dendroctonus ponderosae* is the most damaging insect pest of *Pinus contorta* in western North America,[18] and outbreaks appear to be increasing in response to climate change[19–21] and existing forest conditions. In the western United States, stands in age classes of 60–120 years, and with densities >400 stem/ha, tend to be highly susceptible to *Dendroctonus ponderosae*.[22]

In the past decade, we have witnessed unprecedented levels of tree mortality attributed to *Dendroctonus ponderosae* outbreaks across much of western North America. An ongoing outbreak in British Columbia, the largest outbreak ever documented, affected 11 million hectares in 2007[23] and has since grown to encompass >17.5 million hectares.[24] In the United States, the outbreak impacted >9 million hectares.[25] Although *Dendroctonus ponderosae* is an important part of the ecology of these forests, extensive levels of tree mortality resulting from outbreaks may have undesirable impacts, for example, negatively affecting aesthetics, fire risk and severity, recreation, timber production, and real estate values, among many other factors.

Management of *Dendroctonus ponderosae*

During *Dendroctonus ponderosae* outbreaks, federal, state, and industrial landowners conduct more frequent aerial detection surveys.[26] Infestations can easily be spotted from low-flying aircraft as polygons of red and fading trees. If *Dendroctonus ponderosae* populations are deemed sufficient to warrant control, a continuous program of ground surveillance and aerial surveys is often initiated. A number of risk and hazard rating systems are available to predict the susceptibility of a given stand to *Dendroctonus ponderosae*,[27–31] and chemical-,[32] silviculture-,[22] and semiochemical-based control methods[4] have been developed.

Semiochemical Modulation of Dendroctonus ponderosae *Mass Attacks*

Like many bark beetles, *Dendroctonus ponderosae* uses a complex system of chemical communication during host location, host selection, and host colonization.[33,34] The initiation of gallery formation induces females to produce an aggregation pheromone composed of *trans*-verbenol and *cis*-verbenol.[35,36] At the same time, the host tree releases α-pinene,[37] myrcene, and terpinolene[34,38,39] in response to the attack, which increases the attractiveness of the target tree.[34,36,37,40] The aggregation pheromone recruits other pioneering females to the target tree and induces them to bore into the bark.[41,42] *exo*-Brevicomin is produced by both sexes and appears to be attractive at low concentrations and inhibitory at higher concentrations.[43,44] *cis*-Verbenol, produced by female *Dendroctonus ponderosae,* has been shown to increase the attraction of conspecific females to *exo*-brevicomin, but its effect is less than that of *trans*-verbenol.[36]

Successful host colonization depends on recruiting a minimum number of *Dendroctonus ponderosae*[33,45] to attack the tree and overcome its defenses,[46] a process that is completed within 2–3 weeks. As the abundance of colonizing male *Dendroctonus ponderosae* increases, levels of male-secreted *exo*-brevicomin and frontalin increase[34,47–51] while concentrations of the aggregation pheromones *trans*- and *cis*-verbenol and host monoterpenes decline.[40] This reduces the attractiveness of the target tree and commences latter stages of tree colonization. The secretion of 2-phenylethanol by males[44] and the release of 1-octen-3-ol by females[52] may further reduce attraction to the target tree.

Autoxidation of α-pinene to *trans*- and *cis*-verbenol and then to verbenone,[53] primarily by intestinal and gallery-inhabiting microbes within both sexes of *Dendroctonus ponderosae*,[54,55] inhibits additional *Dendroctonus ponderosae* from infesting the target tree. This inhibition is necessary for reproduction, because limiting the number of infesting beetles increases the likelihood of brood survival.[56] Although the attractant *trans*-verbenol is still being secreted by the beetles in the infested tree, the attracted beetles are repelled from the focus tree by verbenone[57–61] and *exo*-brevicomin.[43] These newly arriving *Dendroctonus ponderosae* then reorient to adjacent trees, where the cycle of colonization may be repeated.[62]

Pheromone-Based Strategies to Manage Dendroctonus ponderosae

Pheromone-based strategies examined for the management of *Dendroctonus ponderosae* involve aggregation pheromones deployed in trap-out, trap-tree, or concentration approaches,[63–65] and the use of antiaggregants to interrupt colonization of hosts.[4,65–75] Semiochemically driven push and push–pull strategies have been proposed[65,76,77] and assayed,[77] but the push-only strategy is regarded to be preferable given that it is much less expensive and simpler to implement than push–pull.[77]

Verbenone

Verbenone is produced in vivo by some insects and is also found in a variety of plants including a wide range of angiosperms.[78–85] It has been approved by the Food and Drug Administration as a food additive[86] and is a constituent of strawberry, raspberry, dill, rosemary, and spearmint flavor mixtures used in the food industry.[87–89] Because verbenone is naturally occurring and can be effective for reducing levels of tree mortality attributed to *Dendroctonus ponderosae* in several tree species,[90] it is currently approved by the U.S. EPA as a biopesticide for use in forestry. Formulations currently registered include pouches (several registrants), Disrupt Micro-Flake® VBN, Disrupt Bio-Flake® VBN, and Disrupt Bio-Dispenser BB (Hercon® Environmental).

Although verbenone has been studied extensively with the goal of protecting individual trees and stands from mortality attributed to *Dendroctonus ponderosae*,[90] results have been inconsistent, a shortcoming that has negated the widespread adoption of verbenone as a management tool for *Dendroctonus ponderosae*. In some cases, it is likely that consistent and acceptable efficacy was not achieved because of shortcomings in the formulation of verbenone. For example, a polyolefin bead formulation was shown to release inadequate levels of verbenone for an inadequate length of time,[91] resulting in inconsistent field results.[92] In another example, a bubblecap formulation was ineffective in protecting individual *Pinus contorta*, likely due to insufficient release of verbenone.[93] Today, although most pouch formulations have been demonstrated to release adequate levels of verbenone for adequate periods,[94,95] they are applied at relatively low densities (typically, <124 U/ha), which limits coverage. It is possible that a high density of long-lasting point sources would provide better dispersal of verbenone and would better simulate the natural release of verbenone in a forest stand, thus ensuring greater efficacy than that achieved by larger dispensers, such as pouches.[96] Furthermore, pouches are manually applied to the bole of each tree. They are not amenable to mechanization of application and require retrieval from the field after treatment, making them difficult to adopt in large-scale programs due to the associated labor costs.

SPLAT Verb

An ideal formulation of verbenone for management of *Dendroctonus ponderosae* should (1) provide monolithic reservoir-type release at adequate rates (>50 mg/day) for an adequate period (~3 months in *Pinus contorta*); (2) allow for the application of a relatively high density of point sources per unit area (>375 ha); (3) allow for application by manual or mechanized means using conventional

equipment; and (4) be fully biodegradable (within ~12 months), allowing the formulation to be left in the field. Additional desirable attributes include (1) ease of flow from the storage reservoir to the point of application (this would ensure that it could be manually or mechanically applied by ground or aerial equipment); (2) good adhesion as applications must remain fixed to the intended target for several months regardless of weather conditions (it must be rainfast and ultraviolet [UV] protected); (3) eco-friendly; and (4) unobtrusive (the formulation should be almost invisible to the general public). As mentioned earlier, ISCA Technologies has developed a SPLAT formulation that addresses these desirable characteristics. SPLAT is inexpensive, flowable, rainfast, and UV protected and adheres to most surfaces, sticking particularly well to pine bark (C. J. Fettig, A.S. Munson, and A. Mafra-Neto, et al. unpublished data). It can be stored in regular containers and can be cleaned easily using nothing more than soapy water. Applications of SPLAT to plants have no phytotoxic effects. Point sources eventually fall to the ground and biodegrade without consequence. Most importantly, the release rate of most active ingredients from SPLAT is surprisingly constant, decreasing slowly over a period of weeks to months depending on the formulation. The amorphous and flowable quality of this highly adaptable product allows for easy transition from small-scale manual applications (single trees) to large-scale mechanized applications (campgrounds, stands, etc.).

Chemical Stability of SPLAT Verb under Field Conditions

SPLAT Verb was designed to release verbenone over a sustained period (8–24 weeks, depending on dollop size) at levels that disrupt the aggregation behavior of *Dendroctonus ponderosae*, thereby preventing or reducing its number of attacks and subsequent levels of tree mortality. A weakness of some other formulations of verbenone is that under direct sunlight verbenone may be photoisomerized to chrysanthenone,[97] a compound with no known effects on bark beetle behavior. Although conventional UV-reflecting pouch release devices contain UV stabilizers that scavenge UV-generated radicals, Fettig et al.[94] indicated that trace amounts of filifolone, a thermal- or photorearrangement product of (+)-chrysanthenone,[98] were present in pouches deployed for several weeks in ponderosa pine, *Pinus ponderosa* Dougl. ex Laws., forests in California.[94] Conversely, data from rigorous analytical chemistry of SPLAT Verb dollops aged up to 12 months in *Pinus contorta* forests in Idaho and Wyoming indicate that this product protects verbenone extremely well. That is, it was only after the dollops were aged for >12 months in the field that we detected the first traces of chrysanthenone (A. Mafra-Neto, C. J. Fettig, and A. S. Munson, et al. unpublished data), and this was found in only one sample. In addition to assays in forests, we also aged dollops of SPLAT Verb in Riverside, California, which has a warm and sunny climate. Our results indicate that even in Riverside isomerization of verbenone to chrysanthenone did not occur in the SPLAT Verb formulation and therefore would likely not represent a substantial concern in areas where SPLAT Verb is likely to be most commonly used (e.g., the Rocky Mountains). It is important to note that these and similar analyses do not address changes in the chemical stability of verbenone once released into the active airspace, which may also influence levels of inhibition, but over which we have no control (Figure 16.3).

Fieldwork

Protecting Individual Pinus contorta *from* Dendroctonus ponderosae

Study 1

A pilot study was conducted in 2011 on the Bridger-Teton National Forest, Wyoming. Trees treated with SPLAT Verb and untreated controls (n = 21 SPLAT Verb, due to limited quantities available; n = 30 untreated control) were confirmed to be uninfested by *Dendroctonus ponderosae* prior to treatment. Four large dollops of SPLAT Verb were applied at approximately 3 m in height

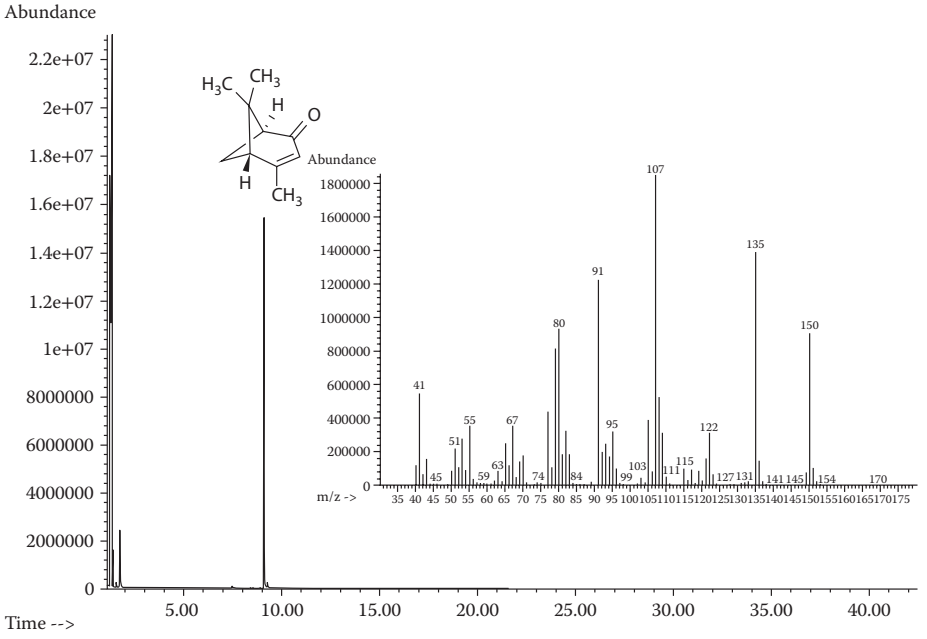

Figure 16.3 A representative analysis using gas chromatograph and mass spectrometer of the solvent extraction of a single SPLAT Verb dollop that was aged for 1 month under direct sun exposure in Riverside, California. The trace shows a single large peak, characterized as verbenone.

on the tree bole, using a Graco 15:1 automotive-style pneumatic-grease pump (Graco, Minneapolis, Minnesota) with a 5-m hose attached to a spray nozzle. The pump was powered by a portable gasoline-driven air compressor. All equipment was housed in the bed of a John Deere Gator™ (Figure 16.1). A total of 15 trees received approximately 32 g of (–)-verbenone per tree (~533 g of SPLAT Verb), and the remaining 6 trees received approximately 39 g of verbenone per tree (~650 g of SPLAT Verb). We recognized that these rates are higher than operationally applied for individual tree protection (typically, one or two 7-g verbenone pouches are used), but timing precluded us from determining application rates until after treatments were implemented. Treatments were applied in mid-July (13.9°C–25.6°C, 33%–65% relative humidity, winds <5 km/h) approximately 7–10 days after the initiation of *Dendroctonus ponderosae* flight in the area. A 10-minute rainfall occurred immediately after application. All SPLAT Verb–treated and –untreated control trees were baited with one *Dendroctonus ponderosae* tree bait (Contech, Delta, British Columbia, Canada) positioned at a northern aspect approximately 2.4 m in height on the tree bole. Untreated control trees were baited before those treated with SPLAT Verb, an important caveat but one we feel had little impact given that only limited flight (~3 weeks) occurred before the baiting of SPLAT Verb–treated trees. Baits were removed from all experimental trees approximately 30 days after baiting, at which time all trees treated with SPLAT Verb were visually evaluated for dollop integrity. Preliminary assessments of tree health were performed in September 2011 by visually examining trees for signs of *Dendroctonus ponderosae* attack. Final evaluations were based on the presence (dead) or absence (alive) of crown fade in June 2012.

Only one SPLAT Verb–treated tree showed signs of *Dendroctonus ponderosae* attack, whereas 83.3% of untreated control trees suffered mass attack (boring dust and/or pitch tubes encircling the tree bole) by *Dendroctonus ponderosae* at levels high enough to suggest that mortality was imminent. Evaluations of tree mortality in the following year indicated that SPLAT Verb provided 100% tree protection, whereas only 6.7% of the untreated control trees survived (Table 16.1, Figure 16.4).

Table 16.1 Preliminary Study of the Effectiveness of SPLAT Verb for Protecting Individual *Pinus contorta* from *Dendroctonus ponderosae* Attack, Bridger-Teton National Forest

Treatment	*N*	Percentage of Trees Alive
SPLAT Verb	21	100.0
Untreated control	30	6.7

Figure 16.4 (See color insert.) Crown fade (yellow-brown needles) in untreated control trees used in SPLAT Verb pilot study. Only 6.7% of the untreated control trees were without signs of crown fade at the time when final evaluations were made (June of the field season following treatment).

Study 2

Based on the preliminary results obtained from the pilot study (Study 1), a second study was initiated in the same area to confirm the effectiveness of SPLAT Verb for the protection of individual *Pinus contorta*. A total of 30 randomly selected *Pinus contorta* were treated with SPLAT Verb using a caulking gun (Newborn XLite, Newborn Brothers Co., Inc., Jessup, Maryland) (Figure 16.5). Four dollops [total 7 g of (–)-verbenone per tree] were applied at approximately 3-m. An additional 30 Pinus contorta were randomly selected as untreated controls. All experimental trees were confirmed to be uninfested by *Dendroctonus ponderosae* prior to treatment and baited with one Dendroctonus ponderosae tree bait (Contech) on the northern aspect at approximately 2.4-m height for 113 days.

Preliminary assessments of tree health were performed in September 2012 by visually examining the trees for signs of *Dendroctonus ponderosae* attack (as in Study 1). None of the SPLAT Verb–treated trees showed signs of mass attack (Table 16.2), whereas 93% of untreated control trees suffered mass attack. We also evaluated all host trees within a 11-m radius of each experimental tree for signs of attack (Table 16.2). None of the trees surrounding SPLAT Verb–treated trees suffered mass attack by *Dendroctonus ponderosae*, whereas 61 trees surrounding untreated controls exhibited mass attack, suggesting that the zone of inhibition surrounding each SPLAT Verb–treated tree was at least 11 m. This represents a much larger area than that previously reported for other

Figure 16.5 Manual application of SPLAT Verb using a caulking gun. Each treated tree received 7 g of verbenone.

Table 16.2 Effectiveness of SPLAT Verb for Protecting Individual Neighboring *Pinus contorta* from *Dendroctonus ponderosae* Attack, Bridger-Teton National Forest

Treatment	Treated Trees	Trees Within 11-m Radius of Treated Tree	
	Not attacked (%)	Mass attacked[a] (%)	Number mass attacked[a]
SPLAT Verb	100	0	0
Nontreated	6.7	93.3	61

Note: Trees were evaluated 3 months after treatment for signs of attack.
[a] Entire circumference of bole attacked.

formulations of verbenone. For example, one study found that verbenone bubblecaps inhibited *Dendroctonus ponderosae* attraction to baited multiple-funnel traps at a distance <4 m in *Pinus contorta* forests in British Columbia.[99] Similarly, Fettig et al.[94] evaluated a 5-g verbenone pouch and reported similar results for the western pine beetle, *Dendroctonus brevicomis* LeConte, a closely related species, in *Pinus ponderosa* forests in California. No significant differences were observed among *Dendroctonus brevicomis* captures at 0.5, 1, or 2 m from the pouch, but significantly more *Dendroctonus brevicomis* were collected at 4 and 9 m.[94]

Protecting 0.4 ha Plots of Pinus contorta *from* Dendroctonus ponderosae

Although demonstrating the efficacy of SPLAT Verb for the protection of individual trees is important, verbenone is most typically applied to protect small-scale areas (campgrounds). Accordingly, we evaluated SPLAT Verb for small-scale stand protection using 0.4-ha experimental plots on the Caribou-Targhee National Forest, Idaho. Treatments included (1) untreated control, (2) (–)-verbenone pouch (7-g pouch, Contech), and (3) SPLAT Verb (7 g of (–)-verbenone). Verbenone pouches were stapled in an approximately 9.1 × 9.1 m grid (125 U/ha) to the north side of the nearest tree at a height of approximately 2 m. SPLAT Verb was also applied to the north side of trees at a height of approximately 2 m in suitable dollop sizes to achieve adequate coverage and cumulative application rates of 875 g/ha of (–)-verbenone. A complete census of each plot of trees was conducted prior to the treatment interval. Any trees attacked by *Dendroctonus ponderosae* were excluded from

TABLE 16.3 Effectiveness of Verbenone Treatments for Protecting Small Stands of *Pinus contorta* in Montpelier Ranger District, Caribou-Targhee National Forest

Treatment	Number of Trees Mass Attacked[a]
SPLAT Verb (875 g/ha)	8
Verbenone pouch (875 g/ha)	18
Untreated control	50

Note: Trees were evaluated 3 months after treatment for signs of attack.
[a] Total number of trees attacked in five 1-acre square plots per treatment.

subsequent analyses. Treatments were applied before the start of *Dendroctonus ponderosae* flight, as confirmed by the lack of captures in pheromone-baited traps (Contech). In the center of each plot, the nearest tree was baited with a *Dendroctonus ponderosae* tree bait (Contech). Thus, baiting provides a rigorous evaluation of efficacy at the expense of detecting any subtle treatment effects.

Preliminary assessments of tree health were performed in September 2012 by visually examining trees for signs of *Dendroctonus ponderosae* attack (as in Study 1). Each *Pinus contorta* within each experimental plot was evaluated for signs of attack, and the number of mass-attacked trees in all five replicates (0.4 ha plots) was tallied for each treatment (Table 16.3). In all SPLAT Verb–treated plots, only eight trees were mass attacked by *Dendroctonus ponderosae*. A total of 18 trees were mass attacked in the plots treated with the 7-g verbenone pouch, and 50 trees experienced mass attacks in the untreated control plots.

Summary: SPLAT Verb

Over several decades, substantial research has focused on the use of antiaggregants, primarily verbenone but also other inhibitory compounds,[15] to disrupt the responses of *Dendroctonus ponderosae* to attractant-baited traps and attractant-baited and attractant-unbaited trees in hopes of developing tactics to reduce levels of tree mortality attributed to *Dendroctonus ponderosae*. Early experiments showed that there was significantly less tree mortality on verbenone-treated plots than untreated plots.[4,90] Later studies yielded inconsistent or ambiguous results over time,[92,100] geographical area,[100,101] outbreak intensity,[72,102] dose,[101,103] or tree species,[4,90] with studies indicating that verbenone is largely ineffective for reducing levels of tree mortality in *Pinus ponderosa*. Progar et al.[90] identified nine factors limiting the effectiveness and utility of semiochemical treatments for the management of *Dendroctonus ponderosae*, at least three of which are relevant to the performance of SPLAT Verb compared to conventional release devices: (1) enhanced chemical stability, (2) an increased range of inhibition, and (3) the ability to achieve more uniform coverage per unit area. Furthermore, dollops of SPLAT Verb biodegrade rapidly and, therefore, do not need to be retrieved from the field as do conventional release devices. This potentially represents significant labor cost savings in the execution of operational *Dendroctonus ponderosae* suppression campaigns (A. S. Munson, unpublished data). Although development of SPLAT Verb uses in forests is ongoing, we believe this product represents an important contribution to the relatively few effective tools that forest health specialists have available to protect trees from mortality attributed to *Dendroctonus ponderosae*.

ASIAN CITRUS PSYLLID, *DIAPHORINA CITRI*

The U.S. citrus industry faces a serious threat from the invasive *Diaphorina citri*, which was first established in the United States in 1998[104–106] in Miami. Although feeding and/or oviposition by these insects may result in direct damage to plant tissue,[107] the primary impact of this invasion is the psyllid's role in the transmission of Huanglongbing (HLB), one of the world's most serious diseases of citrus[108] (Figure 16.6 and Figure 16.7). Also known as citrus greening, HLB is associated with the presence of the pathogen *Candidatus Liberibacter* spp.[108] With the recent invasion of California by

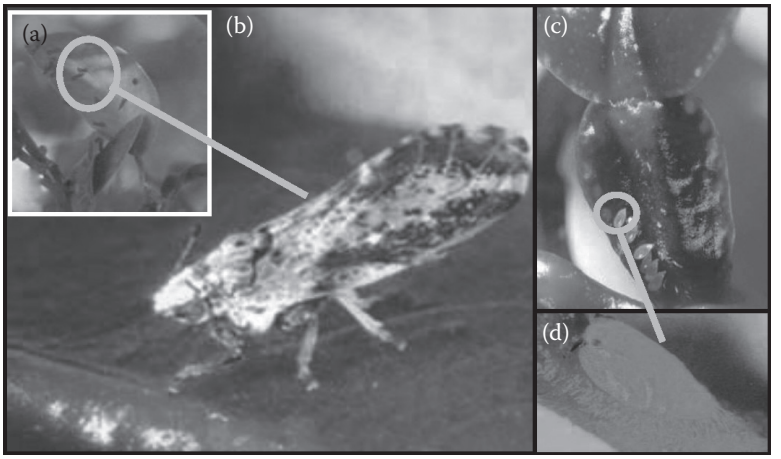

Figure 16.6 **(See color insert.)** *Diaphorina citri*, Asian citrus psyllid, the vector of the *Candidatus Liberibacter* spp. that causes the devastating and irreversible Huanglongbing (HLB), or citrus greening disease. Today, Asian citrus psyllid (ACP) is present in every citrus-producing state in the United States. ACP is a very effective vector of HLB because *Candidatus Liberiobacter* grows extremely fast inside the infected nymphs (c, d), amplifying their presence and thus increasing their hosts' ability to vector HLB as adults. Thus, the effective control of *Diaphorina citri*, and keeping population at extremely low levels, is very important to citrus growers. SPLAT ACP Repel represents an important tool for the management of *Diaphorina citri* (a, b).

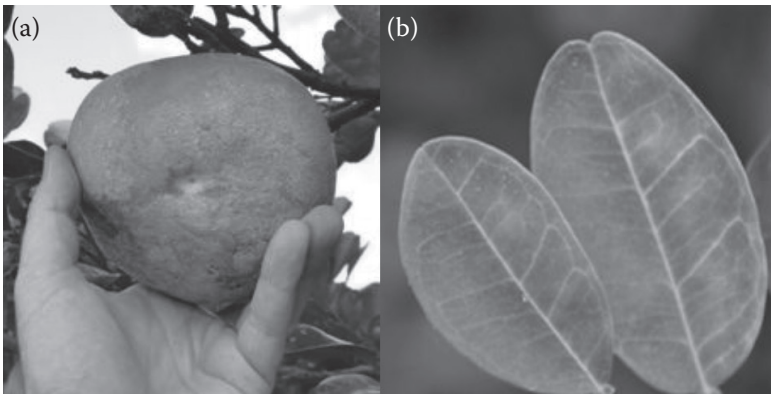

Figure 16.7 **(See color insert.)** Huanglongbing (HLB) is one of the most destructive citrus diseases worldwide. (a) A pomello fruit showing classic symptoms of infection including stunted growth and an irregular shape. The fruit itself is not palatable. (b) A leaf showing the corky veins and the unbalanced chlorosis, characteristic of HLB infection.

Diaphorina citri, this pest is now present in all major citrus-producing states in the United States. The HLB pathogen invariably follows the vector as it expands its range, with trees showing signs of the disease approximately 1–3 years after infection. Citrus trees infected with HLB produce unmarketable fruit and will ultimately die from the disease.

In 2005, the first detection of HLB-infected citrus trees in Florida[109] triggered a concerted effort to detect and remove HLB-infected trees, which has since become a monumental task due to the high rate of HLB infection. This detection and removal program peaked in 2009, when >1 million HLB-infected citrus trees were removed from Florida groves. As the prevalence of HLB increased in this area, growers began providing supplemental nutrition to their citrus plants. HLB reduces the availability of nutrients to the infected tree, causing a rapid decline in its overall health.

Careful supplementation of nutrients does not cure the disease but enables continued fruit production despite HLB infection. This, in turn, allows growers to maintain their productivity during the HLB epidemic. However, growers discovered that supplemented plants produce more and better fruit if they are not reinfected with HLB; so effective suppression of *Diaphorina citri* populations remains crucial, even in orchards where most of the plants are already infected with HLB and are being treated with supplemental nutrition.

Management of *Diaphorina citri* and HLB currently relies on high inputs of broad-spectrum insecticides, which almost certainly have adverse effects on natural enemies that might otherwise contribute to control of *Diaphorina citri* and other citrus pests.[107,110,111] Many stakeholders believe that this strategy is unsustainable, and there is growing concern over *Diaphorina citri*'s recently developed resistance to these insecticides.[112–114] There is an urgent need to develop novel and effective tools to manage *Diaphorina citri*. Furthermore, in response to consumer demand, citrus growers seek low-risk alternatives to broad-spectrum insecticides that can be applied using equipment they already possess. Although HLB is not completely successfully managed in any region of the world where the disease and the vector coexist, the most successful management efforts involve a combination of clean nursery stock, prompt removal of inoculum, and aggressive insecticides sprays against the psyllid.[109]

Solution: Develop an Effective Natural Repellent to Control *Diaphorina citri* and Mitigate Huanglongbing

Based on recent innovations in research that have shown great potential for use in *Diaphorina citri* management, we developed a botanically derived repellent, identified from guava leaves.[105] This compound, dimethyl disulfide (DMDS), was formulated into SPLAT and used to successfully repel *Diaphorina citri* from citrus groves.

Botanically Derived Semiochemicals: Repellents

Botanical insect repellents and insecticides have a prominent role in agricultural pest management. Classic examples include the neem tree–derived insecticide, the antifeedant, azadirachtin and the tobacco-derived insecticide nicotine. *Diaphorina citri*'s abundance in citrus groves is likely influenced by factors like host-plant suitability, mate-finding dynamics, and the presence of surrounding nonhost plants. Numerous chemical and environmental cues can also impact the behavior of adult insects, particularly those signals produced by host and nonhost plants. General models for host-searching behavior by herbivorous insects suggest that host location is a sequential process during which, at each successive step, the physiochemical signals associated with the plant may determine acceptance or rejection by the insect (for feeding or oviposition). Insects' detection of these cues, specifically by Hemipteran species, relies on a variety of sensory modalities, including vision, olfaction, and mechanoreception. Interfering with the host-finding process of the HLB vector is one potential method for reducing its spread. Development of an effective *Diaphorina citri* repellent formulation could improve citrus pest management in many ways. For one, a repellent formulation targeted specifically toward *Diaphorina citri* would not harm biological control agents, which are virtually eliminated from citrus groves by the frequent spraying of broad-spectrum insecticides. Adding an effective botanical repellent to a citrus management program could lessen the number of insecticide applications needed to achieve control, improve insecticide rotation, and reduce the potential for insecticide resistance.

The volatiles released by common guava, *Psidium guajava* L., have garnered intense interest because a recent report showed that guava grown in proximity to, or intercropped with, citrus had a repellent effect toward *Diaphorina citri*.[115] Citrus groves interplanted with guava were devoid of *Diaphorina citri* infestation, whereas nearby citrus groves without guava were heavily infested.[116]

Given that the presence of guava appears to reduce *Diaphorina citri* populations and subsequent incidence of HLB, the cause of this effect was investigated. Leaf volatiles released by white guava leaves, both crushed and intact,[105] collected using static headspace solid-phase microextraction (SPME), were identified using gas chromatography with pulsed flame photometric detection and gas chromatography–mass spectrometry. Leaf volatiles from four common guava cultivars were examined via a similar process to identify the potential components responsible for guava's repellency effect.[105] A total of 54 leaf volatiles were isolated by linear retention index (LRI) and mass spectrometry (MS) data in the crushed guava leaf headspace, including seven sulfur volatiles: hydrogen sulfide, sulfur dioxide, methanethiol, dimethyl sulfide (DMS), DMDS, methional, and dimethyl trisulfide (DMTS).[105] Identifications were based on matching LRI values on ZB-5, DB-Wax, and porous-layer open tubular columns and MS spectra in the case of DMDS and DMS. The DMDS is formed immediately after the guava leaf is crushed and becomes the predominant headspace volatile within 10 minutes and is an insect-toxic, defensive volatile produced only by wounded guava, and it is not found in citrus leaves. Consequently, DMDS was selected as the component most likely to be responsible for the repellent effect of guava against *Diaphorina citri*. Laboratory investigations subsequently proved that DMDS from guava was highly repellent toward adult *Diaphorina citri*. In Y-tube bioassays, DMDS- and guava-related compounds showed toxic effects to *Diaphorina citri*, resulting in knockdown of exposed adults.[117,118]

Laboratory Work with Botanical Volatiles

We examined the effect of a series of potential botanical repellents on *Diaphorina citri* in behavioral assays. Repellents were also evaluated in combination with citrus odors to verify their repellency in the presence of these attractive compounds. Finally, we examined the repellent effects of a select number of plant volatiles formulated in SPLAT.

Treatments tested included combinations of whole plants, leaves, and leaf extracts. Valencia orange, *Citrus sinensis* L., plants were grown in 3.78-L pots in a temperature-controlled greenhouse. Plant samples were composed of 10-week-old whole plants or 2-g samples of fresh leaves. DMDS (≥98% purity), DMTS (≥98.5% purity), and allyl methyl sulfide (AMS) (≥98% purity) were obtained from Sigma-Aldrich, St. Louis, Missouri. Allyl methyl disulfide (AMDS) (≥98% purity) and allyl disulfide (ADS) (~80.0% purity) were obtained from Frutarom Ltd., Billingham, United Kingdom, and Penta Chemical Company, United States.

A custom-designed two-port divided T-olfactometer (Analytical Research Systems, Gainesville, Florida) was used to evaluate behavioral responses. The olfactometer consisted of a 30 cm–long glass tube with 3.5 cm internal diameter that is bifurcated into two equal halves with a Teflon strip, forming a T-maze. Each half served as an arm of the olfactometer, enabling *Diaphorina citri* to choose between two potential odor fields. Its arms were connected to odor sources placed in guillotine volatile collection chambers or SPME chambers, through Teflon glass tube connectors. Its olfactometer was housed within a temperature-controlled room and positioned vertically under a fluorescent 900-lx lightbulb within a 1.0 × 0.6 × 0.6 m fiberboard box to achieve uniform light diffusion. This position took advantage of the negative geotactic and positive phototactic responses of *Diaphorina citri*. The olfactometer inlet adapter was covered with black cloth to facilitate insect movement toward odor sources. An odor source was randomly assigned to one of its arms at the beginning of each bioassay, and it was reversed after every 30 insects to eliminate positional bias.

A minimum of 120 adult female *Diaphorina citri* were examined per treatment combination (four replications with 30 psyllids per replication). *Diaphorina citri* females were released individually into the inlet adapter at the base of the olfactometer and given 300 seconds to show a behavioral response by entering either olfactometer arm. The number of adults entering the treatment arm or control arm or remaining in the inlet adapter (release port) or below the T-maze division was recorded. A treatment or control arm choice was recorded when an insect moved into either

olfactometer arm by crossing the division in the T-maze olfactometer. A release arm choice was recorded when an insect remained in the release port or below the T-maze division. All experiments were conducted at 26°C ± 1°C and 60% ± 2% relative humidity. The olfactometer and connecting tubes were thoroughly cleaned with 2% soap solution and baked at 93.3°C between treatment runs.

Behavioral bioassays with synthetic compounds. Allyl methyl trisulfide, diallyl trisulfide, DMTS, ADS, AMDS, DMDS, diallyl sulfide, and AMS were evaluated for their effect on *Diaphorina citri* behavior at 0.25%, 0.5%, and 1.0% concentrations, both individually and in combination with citrus leaves. The chemical samples were dissolved in 1 mL ethylene glycol (EG), to slow the release rate of the volatile sulfur compounds during the bioassays,[117,118] and pipetted onto a 5-cm Richmond cotton wick (Petty John Packaging Inc., Concord, North Carolina). The treated wick was then wrapped in laboratory tissue and placed in SPME chambers. The control treatment contained cotton wicks impregnated with 1 mL EG only. For evaluations of chemicals in the presence of citrus odors, approximately 2 g of fresh citrus leaves were placed in both chemical treatment and control arms of the olfactometer.

For assays in which putative repellent treatments were presented in the T-maze olfactometer with or without citrus and versus clean air, the number of *Diaphorina citri* remaining at the release point and not entering the olfactometer was compared between treatments using one-way analysis of variance, followed by Tukey's honest significant difference test ($p < .05$). For psyllids leaving the release arm, the number of psyllids choosing the control arm versus the treatment arm was compared with chi-square (χ^2) analysis at $p < .05$. The data from all four replicates were combined for the χ^2 analysis.

Significantly more *Diaphorina citri* remained at the release point in treatments where DMTS (at 0.25% concentration) was copresented with clean air or citrus odors than when clean air and citrus were presented simultaneously in both arms of the olfactometer (Figure 16.8a). No other synthetic sulfur chemical yielded significant differences from the control treatment (clean air) at the 0.25% concentration with respect to the number of *Diaphorina citri* leaving the release point. Significantly more psyllids entered the control arm than the treatment arm when 0.25% DMTS was compared with clean air (Figure 16.8a), indicating that DMTS exerts a high level of repellency on *Diaphorina citri* females.

Significantly more *Diaphorina citri* remained at the release point in treatments where DMTS, DMDS, AMDS, or ADS (all at 0.50%) was copresented with clean air or citrus odors compared to clean air alone, or citrus odor was simultaneously presented in both arms of the olfactometer (Figure 16.8b). The percentages of psyllids not moving from the release point for DMTS versus citrus and DMTS versus clean air ranged between 79% and 89%, respectively, whereas the percentages of psyllids not moving from the point of release for disulfides (DMDS, AMDS, and ADS) ranged between 41% and 60% (Figure 16.8b). Significantly more *Diaphorina citri* chose the arm with clean air compared to DMDS or AMDS and with citrus odors compared to DMDS or AMDS. The percentages of psyllids moving from the release point to DMTS versus clean air and DMTS versus citrus were 11% and 21%, respectively.

Significantly more *Diaphorina citri* remained at the release point in treatments where DMTS, DMDS, AMDS, or ADS (all at 1.0%) was copresented with clean air or citrus odors than when clean air alone or citrus was simultaneously presented in both arms of the olfactometer (Figure 16.8c). The percentages of *Diaphorina citri* not moving from the release point for DMTS versus citrus and DMTS versus clean air ranged from 84% to 92%. The percentages of psyllids not moving from the release point in treatments with disulfides (DMDS, AMDS, and ADS) ranged from 62% to 82%. Percentages of *Diaphorina citri* not moving from the release point were statistically equivalent for trisulfides and disulfides (Figure 16.8c). There were no differences between the number of *Diaphorina citri* entering the olfactometer arm containing AMS versus clean air. Significantly more psyllids remained in the release arm when the 0.25% blend of DMTS + DMDS versus clean air was

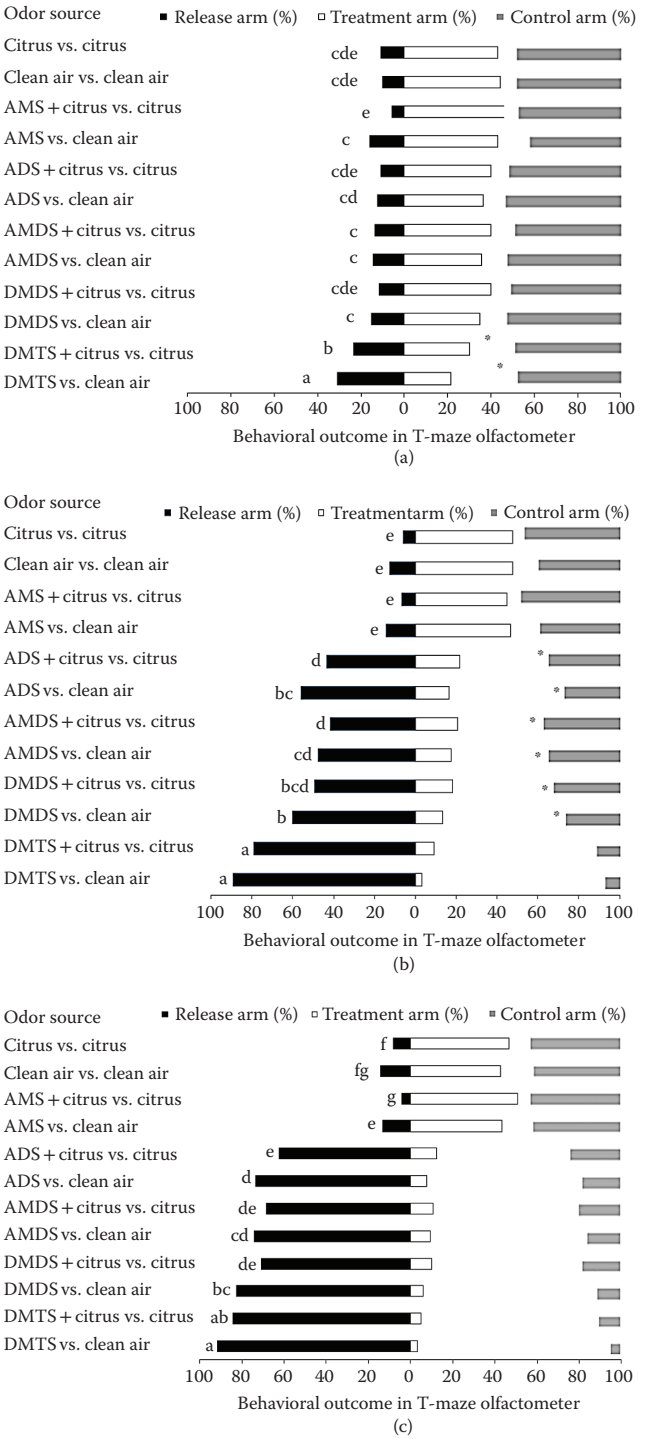

Figure 16.8 Responses of female adult *Diaphorina citri* presented with sulfur volatiles at (a) 0.25%, (b) 0.5%, or (c) 1.0% concentrations with or without citrus odors. Allyl methyl sulfide (AMS), allyl disulfide (ADS), allyl methyl disulfide (AMDS), dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS). Black bars followed by same letters are not significantly different (Tukey's honest significant difference, $p < .05$).

presented than when DMTS alone (0.25%) versus clean air was presented. It is possible, therefore, that blends, instead of single components, could provide optimal repellency to *Diaphorina citri*.

SPLAT ACP Repel

Fieldwork

Field tests of SPLAT formulations releasing DMDS were conducted in Florida in 20-tree blocks of mature sweet oranges, *Citrus sinensis* var. "Valencia," with four replicate blocks per treatment (SPLAT and control). Trees were 12 years old, planted with 3 × 6 m spacing, and averaged 4 m in height. Yellow sticky card traps were used to assess population densities of *Diaphorina citri*. SPLAT ACP Repel was applied at a rate of 6.2 kg/ha, that is, approximately 30 g of SPLAT per tree (six 5-g dollops per tree). This test proved that *Diaphorina citri* population densities can be significantly reduced by the application of DMDS to small plots of citrus (Figure 16.9). A follow-up field trial was performed with SPLAT ACP Repel, containing varying concentrations of the DMDS, and several different additives slowed the release rate of the active ingredient. This resulted in a formulation of SPLAT ACP Repel that provides 5 weeks of repellency toward *Diaphorina citri* in field conditions (Figure 16.10).

Diaphorina citri are known to recolonize citrus groves 1–2 weeks after an application of insecticide, because insecticide residues are relatively short-lived, and *Diaphorina citri* adults are highly mobile.[119,120] This creates the need for a high frequency of insecticide sprays. Some citrus growers in Florida and Brazil have been applying insecticides every 3–4 weeks during the field season to manage *Diaphorina citri*. To evaluate whether the application of SPLAT ACP Repel could help growers reduce the frequency of insecticide sprays, we designed the following experiment to determine whether *Diaphorina citri* recolonization in an area previously treated with insecticide was slowed by the presence of SPLAT ACP Repel compared to those areas receiving insecticide sprays alone.

Using a simple, randomized block design with two treatments, (1) insecticide followed by SPLAT ACP Repel and (2) insecticide only, all treatment plots received a label rate (40.0 oz./ha) application of the pyrethroid insecticide Danitol (Valent USA, Walnut Creek, California) at the start of the experiment to knock down *Diaphorina citri* population. After 2 weeks, half of the

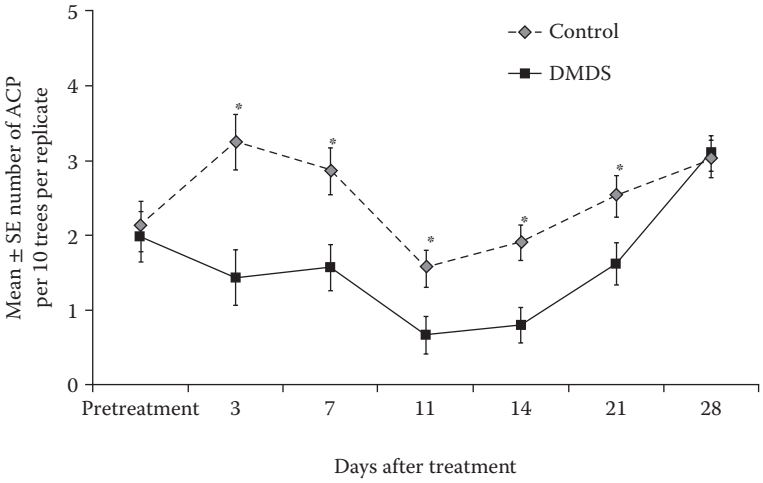

Figure 16.9 Reduction in *Diaphorina citri* populations following treatment with SPLAT containing dimethyl disulfide (DMDS). Results suggest that application of DMDS to an infested orchard can cause existing *Diaphorina citri* populations to disperse away from treated trees.

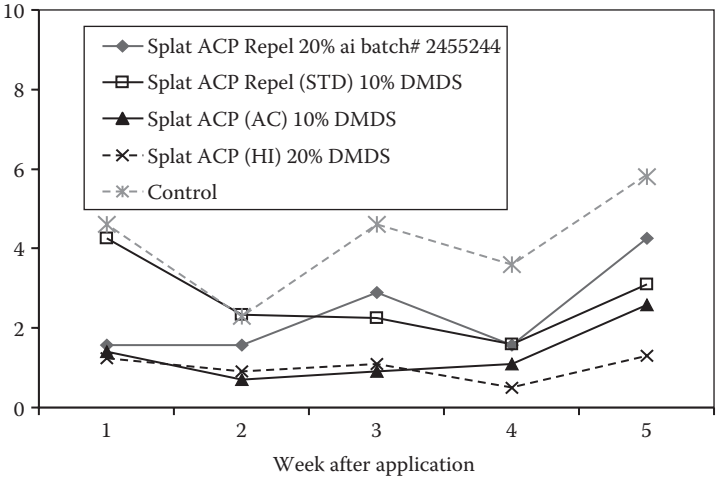

Figure 16.10 Optimization of SPLAT ACP Repel formulations in field testing. SPLAT was formulated with varying concentrations of dimethyl disulfide (DMDS) and with different additives to determine the ideal combination for maximum field longevity.

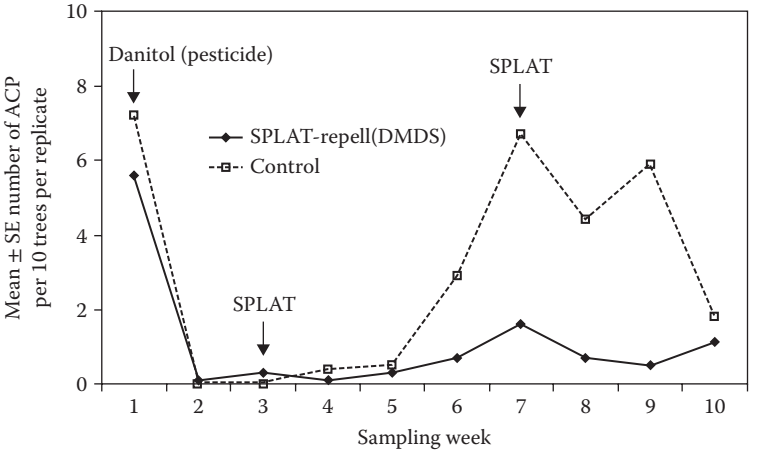

Figure 16.11 Delayed immigration of *Diaphorina citri* into insecticide-treated plots following application of SPLAT with dimethyl disulfide (DMDS). In untreated plots, populations of *Diaphorina citri* began to rebound 6 weeks after treatment, with populations becoming equivalent to pretreatment levels by week 7. In plots that received the SPLAT treatment 2 weeks after the insecticide spray, *Diaphorina citri* populations did not return to pretreatment levels for the entire 10 weeks of study 8.

plots were treated with SPLAT ACP Repel and the remaining half were left untreated. In untreated plots, populations of *Diaphorina citri* began to rebound 6 weeks after treatment, with populations returning to pretreatment levels by week 7. However, in those plots that received the SPLAT ACP Repel treatment 2 weeks after the insecticide spray, *Diaphorina citri* populations did not return to pretreatment levels for the entire 10 weeks of study (Figure 16.11). These data indicate that recolonization by *Diaphorina citri* was impeded by SPLAT ACP Repel and suggest that integration of this product into a *Diaphorina citri* management program has the potential to reduce the number of insecticide sprays needed while suppressing the *Diaphorina citri* population below critical thresholds.

RESULTS

This work indicates that the guava-derived semiochemical, DMDS, is an effective repellent for *Diaphorina citri* at concentrations as low as 10% in SPLAT ACP Repel. When incorporated into SPLAT, the resulting matrix demonstrated the capacity to extend the release period of this extremely volatile compound, prolonging the repellent's efficacy in the field. Field trials have shown that SPLAT ACP Repel with DMDS alone significantly reduces *Diaphorina citri* captures in infested orchards, indicating the potential for this formulation to reduce HLB infection rates in areas treated with SPLAT ACP Repel.

CONCLUSION

SPLAT formulations have been developed to release a variety of compounds, including sex pheromones, kairomones, attractants, phagostimulants, and insecticides. Several mating disruption and attract-and-kill formulations are commercially available. SPLAT Verb and SPLAT ACP Repel represent our first successful attempts at developing repellent formulations and will be registered and marketed in the United States.

Although we have demonstrated that SPLAT Verb is effective for protecting *Pinus contorta* from *Dendroctonus ponderosae*, we surmise this formulation will be useful for protecting other hosts of *Dendroctonus ponderosae* from successful colonization. To that end, additional studies are ongoing. In the future, we plan to evaluate SPLAT Verb against other bark beetles whose primary antiaggregation pheromone is verbenone, such as *Dendroctonus brevicomis* and the southern pine beetle *Dendroctonus frontalis* Zimmermann. Research also continues with SPLAT ACP Repel, specifically integrating it into pest management programs for the management of *Diaphorina citri* in commercial citrus production.

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PART III

Uses

CHAPTER 17

Strategies for Using Personal Protection Products*

Stephen P. Frances

CONTENTS

INTRODUCTION

The use of insect repellents is part of an integrated approach to prevent bites from nuisance and vector mosquitoes and other biting arthropods. This chapter reviews some of the strategies that have been used for personal protection against vectors, especially mosquitoes and discusses basic engineering, repellents in combination with impregnated clothing, insecticide-treated nets, and chemical barriers to insect vectors.

Personal protection measures are the first line of protection against vectors of diseases. Although personal protection measures can reduce the incidence of vector-borne disease, they can rarely eliminate the risk because not all individuals in a community use the measures.[1] In this chapter, the strategies used to reduce nuisance and the potential reduction in the transmission of diseases are discussed. Readers will note that none of the methods or combination of methods is 100% effective because of inconsistency of repellent application, and not all individuals in a community adhere to personal protection.

BASIC ENGINEERING

Humans live in houses or huts made of a variety of materials for shelter from the elements of weather, security, and safety from others. In areas where mosquitoes are a problem, methods have

* This chapter has been approved by the Commander Joint Health (Australia). The opinions expressed herein are those of the author and do not necessarily reflect those of the Australian Defence Force.

been used in construction of homes to minimize the effects of mosquito vectors. At the same time, the houses and villages constructed by humans are sometimes used by mosquitoes for shelter and rest areas, as well as completion of the larval stages in stagnant water that humans also need for their lifestyle.

Work in Africa, where limited funds are available for vector control and personal protection, has focused on low-cost community methods such as bed nets. The use of bed nets will be discussed later. Earlier studies showed that houses and huts in many areas in the African continent were made with local materials and were generally not mosquito proof. During the 1950s, the World Health Organization (WHO) had enthusiastically pursued the use of dichlorodiphenyltrichloroethane (DDT) to treat the inside walls of houses to control *Anopheles* vectors of malaria. The rationale was that *Anopheles* entered the house, bit the sleeping occupants, and then rested on the interior walls of the house. Applying DDT to the inside of these houses resulted in the mortality of resting mosquitoes. The method was used in many countries with financial support from WHO, but because of the development of resistance in many mosquito species to DDT, the practice ceased in many countries during the 1980s.

Some studies in the late 1980s investigated the use of permethrin-treated materials as barriers to the entry of *Anopheles* mosquitoes to huts and houses. In some countries, sisal was treated with permethrin and placed as an insecticide-treated barrier in windows and other areas where *Anopheles* mosquitoes entered the houses.

Impregnated curtains were also used with some success. In Tanzania, permethrin-treated curtains were placed over eaves, which reduced the number of *Anopheles* mosquitoes that fed and survived after entering houses.[2] A study in Burkino Faso in West Africa between July 1987 and May 1989 showed that the use of permethrin-treated curtains reduced the number of malaria episodes, parasitaemia, and splenomegaly during a 22-month period for children 6 months to 6 years old.[3] Although these methods were effective, limited funds were used to supply bed nets and insecticide chemicals to communities throughout the world.

In the last decade, placing barriers in homes in Africa has generated renewed research interest. Early studies showed that the fungus *Beauveria bassiana* (Bals.) Vuill and *Metarhizium anisopliae* (Metschn.) Sorokin had promise in the control of adult *Anopheles*.[4] The studies showed that the fungus provided variable mortality against mosquitoes when applied to dark fabric swatches that were then hung over gaps in the roofs and walls from where mosquitoes entered houses. The mosquitoes contacting the treated cloth became infected and died from the fungal infection later. A study also showed that strains of insecticide-resistant mosquitoes were susceptible to fungal infection.[5] *M. anisopliae* was also used to treat fabrics that were placed in traps to kill adult mosquitoes.

The use of mosquito coils, vaporizer mats, and emanators have been shown to provide protection against mosquitoes, especially within homes. Coils that burn pyrethroid insecticides inhibit biting and feeding of vector mosquitoes, as well as causing knockdown and mortality of some.[6]

REPELLENTS

The use of topical repellents applied to exposed skin is the most important personal intervention against vectors of diseases, and numerous studies have been undertaken to enumerate the protection against biting vectors, especially mosquitoes. The use of repellent active ingredients such as *N,N*-diethyl-3-methylbenzamide (deet), 2-(2-hydroxyethyl)-1-piperidinecarboxylic acid 1-methylpropyl ester (icaridin, picaridin), lemon eucalyptus oil (*p*-menthane-3,8-diol) and ethyl butylacetylaminopropionate (IR3535), as well as several infrequently used synthetic and naturally derived active ingredients are discussed in detail in Chapter 19.

An important consideration in the use of topical repellents by individuals within communities is the cost of obtaining them.[7] This is a limiting factor in the use of repellents in developing countries,

where vector-borne diseases may be endemic and personal disposable incomes are low. This has led to the development of lower cost repellent formulations. For example, a repellent soap formulation containing 20% deet and 0.5% permethrin was developed in Australia in 1985 (Figure 17.1).[8] It was prepared in small blocks (approximate weight, 70 g) and packed in relatively low-cost greaseproof paper, and was initially priced at $0.25 per piece. This formulation was applied to wet skin, lathered, and the residue was left on the skin surface to dry. Several field trials showed the formulation provided satisfactory protection against mosquitoes in Malaysia,[9] Papua New Guinea,[10] Australia,[11] India,[12] Ecuador, and Peru.[13] This formulation was commercialized and marketed as "Mosbar" in Southeast Asia. A survey of personal protection measures used by inhabitants of East Honiara, Solomon Islands, showed a variety of measures were used by people to protect themselves from potential vectors of malaria.[14] The survey showed 10.3% of respondents used Mosbar and 8.4% used unidentified repellent formulations. The study showed that only respondents who used prophylactic drugs or Mortein (pyrethroid aerosol) had increased protection against malaria.[14]

Another important consideration in the use of topical repellents and other personal protection measures is the user acceptability of the formulation. Several studies have been undertaken to determine what factors affect the use of repellents by individuals and groups.[15]

The availability of personal protection measures against mosquitoes is variable within communities and differences are due primarily to socioeconomic reasons. In most communities, the seasonal increase in the density of mosquitoes is expected to result in an increase in the use of mosquito control and protection products. In Tanzania, Chavasse et al.[16] showed a relationship between mosquito densities obtained from trap collections and the sale of mosquito coils in the shops of Mikocheni, Dar es Salaam. In the Gambia, districts with higher mosquito densities had higher rates of bed net usage.[17] Conversely, a study in southern Tanzania showed that mosquitoes were diverted away from households where the occupants used repellents to households who did not.[18] These authors noted that policy makers should take into consideration results showing vectors diverted from privileged families to those less privileged who may be exposed to nuisance and vector mosquitoes.

Figure 17.1 **(See color insert.)** Tom Simmons, developer of Mosbar, applies the repellent soap to the arm of an Australian soldier in 1986.

CLOTHING

Several studies to evaluate the effectiveness of combinations of repellent and insecticide-treated clothing have been undertaken and are discussed here. The protection of an individual is enhanced by reducing the amount of skin exposed to biting insects and arthropods. In most human communities, clothes are worn, and provide a physical barrier to arthropod bites. In many tropical countries, fewer clothes may be worn, as the use of long sleeves and long trousers may not be necessary, allowing more skin to be exposed to arthropods.

The recommendation to wear long-sleeved shirts and long trousers to protect a person against malaria vectors is moderated by the discomfort of wearing such clothing in warm tropical environments. For short-term visitors, they may decide to protect themselves against vectors, whereas longer term residents are less likely to wear/need long clothing.

The use of insecticide treatment of military clothing with synthetic pyrethroid insecticides, usually permethrin was first suggested in the 1970s. Earlier studies showed that dipping clothes into a water emulsion containing 0.6% of permethrin was toxic to flying and crawling insects and ticks.[19] Schreck et al.[20] showed the optimal concentration of permethrin in fabric to protect against mosquitoes, ticks, and chiggers. They also showed that the insecticide was lost from the fabric following washing, whereas relatively little was lost when clothes were just worn normally. Abrasion of clothing also resulted in limited loss of permethrin from fabric.

Early studies with freshly treated and unwashed clothing in combination with deet repellent on the skin provided the best protection against mosquitoes in a rainforest habitat in northern Queensland, Australia.[21] This method was also shown to provide the best protection against mosquitoes in trials in Thailand[22] and Alaska.[23]

The effect of washing fabric on the persistence of permethrin (Figure 17.2) has been shown in Thailand,[24] Australia, United States,[25] and recently in Europe.[26] Permethrin treatment of military uniforms has been used by U.S. and coalition forces, including Australia, for the last 18–20 years. The first field evaluations showed freshly treated unwashed uniforms in combination with the application of deet to the exposed skin provided the best protection against mosquitoes.[21] Treatment of U.S. battle dress uniforms (BDUs) and Australian Disruptive Pattern Combat Uniforms was achieved initially by dipping or spraying in a water/permethrin emulsion.[27] The U.S. BDUs are also

Figure 17.2 Applying permethrin to military uniforms in Thailand, 1992.

treated by the Individual Dynamic Absorption (IDA) kit. Recently, fabrics have been treated with permethrin by several methods in the factory before manufacture into uniforms.[26,28] The loss of permethrin from fabric is primarily due to washing of the uniform during its use by soldiers.[29] The rate of loss of permethrin from the fabric has been shown to be variable, depending on the method of treatment, number of washes, and method of entomological evaluation.

Earlier studies have evaluated persistence of permethrin in fabric by exposing mosquitoes to treated fabric for the amount of time needed to kill a proportion or all of the arthropods exposed.[26,28,30] However, mosquitoes that are foraging for a blood meal spend a relatively short time on surfaces such as clothing. When fabrics have been freshly treated with permethrin, mosquitoes spend only a few seconds on treated fabric and quickly fly off.[11,31]

The method of application of permethrin to clothing and other fabrics was developed during the last 20 years. Initially, clothes were treated by placing them into an emulsion of permethrin and water, allowing the clothes to become saturated, and then allowing the fabric to air dry. On many occasions, military uniforms were initially treated with permethrin and worn by soldiers who were working in areas where vector-borne diseases occurred. However, the rate of re-treatment of uniforms was variable and protection against vectors was compromised.[32,33] Methods of treating uniforms with permethrin were developed to increase the persistence of permethrin against washing. During the last 5 years, the treatment of fabrics has been conducted in factories before making clothing. The U.S. and European countries have used uniforms treated in this way in recent wars in Iraq and Afghanistan.[33] The main benefit to soldiers is that they are able to carry out their duties with reduced concern about vectors of diseases. Education briefs advise that they should continue to apply deet repellent to exposed skin in areas where vector-borne diseases is a primary concern. The developmental improvements in application methods should allow for fabrics to be treated with permethrin and other pyrethroids to increase the protection of civilian workers and people living in malarious areas.

The use of clothes and fabrics treated with synthetic pyrethroids in civilian communities has been investigated in the last decade. A community-wide study in a refugee camp located in northeastern Kenya compared the use of personal clothes (Diras, Saris, Jalbaabs, Ma'awis, and shirts) and bedding (sheets and blankets) treated with permethrin with untreated clothes and bedding on the rate of malaria infection.[34] The study showed that the use of permethrin-treated clothes and bedding reduced the rate of malaria infection and the mosquito biting rate in huts inhabited by people with treated cloths and bedding. The study also showed that the concept of treating clothes was well accepted by the participants who had no side effects because of the insecticide treatment of clothes or bedding over a 6-month period. The use of permethrin treatment of clothing was shown to provide protection from the tick *Ixodes scapularis* Say in laboratory studies. The authors concluded that do-it-yourself treatment of summer clothing (T-shirts, shorts, socks, and sneakers) significantly reduced tick bites and tick-borne pathogen transmission.[35]

A field assessment of the effects of wearing permethrin-treated uniforms on the incidence of malaria in Thai soldiers was conducted in 1992. The results showed no decrease in malaria over a 6-month period.[24] In this trial, soldiers were not required to wear their uniforms after hours and although they were given topical repellent, they were not required to apply this in the evening or at night. In Colombia, the efficacy of permethrin-treated uniforms for the prevention of malaria and leishmaniasis over a 4-week period was studied.[36] The study showed that soldiers wearing the treated uniforms had an increased protection against both diseases, and the authors concluded that permethrin-treated clothing was recommended for exposure to both diseases for a period of 1–2 months.[36] In contrast, a subsequent study in Iran showed that permethrin-impregnated uniforms were not effective for protection of cutaneous leishmaniasis in Iranian troops.[37] In 2000, 22,000 French troops were deployed on military service to Cote d'Ivoire (Ivory Coast). A study showed that industrial impregnation of permethrin offered some protection from mosquitoes, but not enough to reduce significantly the incidence of malaria among nonimmune troops.[38] The contrast in the results

shown in these trials may be due to the period during which the use of permethrin-treated uniforms were monitored. In a field efficacy trial in Colombia, good protection was shown in a 1- to 2-month period, whereas, in other studies, permethrin-treated uniforms were not effective against malaria in Thailand over 6 months and in Ivory Coast over 1–2 months.

BARRIERS

Using physical barriers as protection against mosquitoes has been reported to have occurred in ancient times.[39] Fishermen working in the Mediterranean Sea sometimes slept under fishing nets to protect themselves from mosquitoes. Mosquito bed nets have been used in the tropics for a long time. During World War II, soldiers operating in the southwest Pacific theatre used bed nets to protect themselves from malaria vectors. In the 1980s, the treatment of bed nets with pyrethroid insecticides gained impetus. The synthetic pyrethroid, permethrin, became available in 1975. In many malarious countries, untreated mosquito bed nets were used despite the presence of holes that allowed mosquitoes to enter the net and bite the sleeping person. Sometimes people would awaken to find many engorged mosquitoes enclosed in the net. Curtis and colleagues showed that treating the net with synthetic pyrethroids reduced the ability of *Anopheles* mosquitoes to fly through holes in the bed nets, thereby increasing the protection provided by nets with holes. Bed nets became damaged and because of the cost of replacement, they continued to be used to provide protection against vectors. In the late 1980s, the use of permethrin-treated bed nets was adopted by WHO as a major intervention to reduce the transmission of malaria in Africa and Asia.

Models of host-seeking processes in the context of local human host availability and elucidating the impacts and mechanisms of pyrethroid-treated bed nets were undertaken.[40] The modeling showed the excitorepellency of pyrethroid chemicals increased the exposure of untreated humans by concentrating mosquito biting on this vulnerable (untreated) group. The model predicted that nets would have a significant impact on transmission of disease among users of bed nets. These results are consistent with the outcomes of many controlled randomized trials. For example, a study in India in military camps showed that deltamethrin-treated bed nets provided an 87% reduction in cases of malaria over a 2-year period where three treatments of nets with deltamethrin were undertaken.[41]

An important consideration in the use of bed nets is that *Anopheles* vectors of malaria bite between dusk and dawn, and biting activity usually begins before people went to bed to be protected by bed nets and screens in houses. The use of personal protection measures during this time before retiring to bed has been investigated, with the use of repellents and protective fabrics being investigated. In a study in Cambodia, Sochantha et al.[42] investigated protection provided by permethrin-treated hammocks using Olyset™ technology against exophagic vectors. The study showed that only 46% protection was provided by the insecticide-treated hammocks against *Anopheles minimus* Theobald, and poor protection was provided against *A. dirus* Peyton and Harrison, *A. maculatus* Theobald, and Culicine mosquitoes. The authors suggested that despite the poor results, the insecticide-treated hammocks could prove effective in protecting forest workers and villagers before bedtime, and it is a valuable tool in areas where artemisinin-resistant malaria parasites are emerging. The use of topical repellents in malaria-endemic areas has been suggested as a method to further reduce the risk of exposure to biting *Anopheles* mosquitoes. A study in Pakistan suggested that a repellent containing deet was popular among Afghan refugees and provided protection from malaria in the early evening.[43] A subsequent study in Bolivia showed that the incidence of malaria in adults was reduced when people used a repellent formulation containing a natural active ingredient (30% *p*-menthane-3,8-diol) in combination with sleeping under permethrin-treated bed nets.[44] In South America, a low-cost repellent containing *p*-menthane-3,8-diol and lemon grass oil provided protection comparable to deet against malaria vectors in Guatemala and Peru.[45]

A possible impediment to the success of bed nets is the development of resistance in mosquito vectors to the pyrethroid insecticides used to treat nets. Resistance of malaria vectors to a range of pyrethroid active ingredients has been shown by some workers in Africa. John et al.[46] showed a significant increase in the knockdown time and mean mortality of *A. gambiae* Giles to net material treated with resmethrin, cyfluthrin, and cypermethrin. The study showed the development of resistance to pyrethroids in *A. gambiae* in western Uganda.[46] Other workers have shown treating nets with a combination of active ingredients are beneficial and can potentially decrease the likelihood of the rapid development of resistance in malaria vectors.[47]

The indoor residual sprays were used successfully to control *Anopheles* mosquitoes and to reduce the transmission of malaria. The use of DDT to treat indoor wall and ceiling surfaces of houses has been advocated since the 1950s, when WHO had hoped to eliminate malaria. Owing primarily to the development of resistance, decreased allowance by householders to allow their homes to be treated, and decreased political will among governments who funded the intervention, the use of indoor residual spraying has decreased dramatically since the 1980s. Despite this, Graham et al.[48] used insecticide-treated tarpaulins for the control of malaria in refugee camps in Afghanistan. More recently, Diabate et al.[49] and Chandre et al.[50] have investigated the use of insecticide-treated plastic sheeting, known as durable lining, as a lining for interior walls and ceilings. The plastic lining is placed in the interior of houses and treated with a contact irritant to prevent mosquitoes from resting inside homes, and potentially causing death among mosquitoes that spend too much time on the plastic surface. Chandre et al.[50] noted that the plastic sheet is pretreated with insecticide in the factory, and retreatment is not required. The use of insecticide-treated plastic sheeting as both a shelter and protection against malaria was conducted in refugee camps in Sierra Leone.[51] This study showed a protective efficacy of 61% under fully lined insecticide-treated plastic sheeting and only 15% in shelter where only the roof was lined. The authors reported improvements in anemia rates when both methods were used, and both methods were considered a convenient, safe, and long-lasting method of malaria control.[51] The use of insecticide-treated plastic sheeting to line shelters used in refugee camps and during humanitarian aid programs provides emergency shelter as well as improved protection against vector-borne diseases and will be used by aid programs in the future.

The application of insecticides and repellents to clothing, other fabrics, and screens has been evaluated and used in several places to minimize contact between people and vectors, especially mosquitoes. During World War II, Allied Forces operating in the southwest Pacific region experienced significant morbidity and mortality from scrub typhus, a disease caused by *Orientia tsutsugamushi* (Hayashi) and carried by trombiculid mite larvae, commonly called chiggers.[52] Studies in Australia and Papua New Guinea during 1943 showed that dibutyl phthalate applied externally to military shirts and trousers provided excellent protection against chiggers. A concerted effort was undertaken to show soldiers the best method of application and to encourage the use of this chemical among the allies; and during 1944, there was an 80% decrease in the incidence of scrub typhus.[53] This is one of the few examples where the use of a repellent chemical has had a measurable effect on the incidence of disease.[54] This method was subsequently adapted for use in communities after the war as protection against chiggers and scrub typhus.

Bifenthrin[2-methylbifenyl-3-ylmethyl(*Z*)-(1*RS*)-*cis*-3-(2-chloro-3,3,3-trifluroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylate] is a non-α-cyano pyrethroid, and is used against a range of agricultural pests and as an insecticide treatment for mosquito bed nets.[55] The chemical has a relatively low irritant and knockdown effect compared with permethrin and deltamethrin. Bifenthrin causes a higher mortality by allowing mosquitoes to rest on treated surfaces for longer periods.[56] In a study in India, the rate of entry of mosquitoes into rooms containing bifenthrin- and lamdacyhalothrin-treated bed nets was fewer than those entering rooms containing untreated bed nets.[57]

Military and civilian tents are commonly used to house refugees, displaced persons, and aid workers following natural disasters and civilian unrest. These tents may be in place for extended periods when the occurrence of vector-borne diseases such as malaria and dengue may increase.

Following rain, the folds in these tents provide breeding sites for *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse), the vectors of dengue fever (Figure 17.3). For example, a survey of Australian military peacekeeping installations in Timor-Leste in 2001 found 41 containers with dengue vectors, 22 (53.7%) of which were from water found in tent folds (R. D. Cooper, unpublished data). As a control measure, military tent fabrics have been treated with synthetic pyrethroids, such as permethrin and bifenthrin, to reduce the entry and of mosquito bites.[58,59] The effects of the insecticide on the eggs and larvae of *Aedes aegypti* when applied to tent fabric showed eggs and larvae did not develop, and the ability of adults to obtain a blood meal after exposure to treated fabric was significantly reduced.[60] The treatment of the inner walls of tents with permethrin reduced the nuisance of mosquitoes and probably invasive pests,[59] and has been shown to provide good protection against vectors of malaria.[61] The treatment of Australian military tents with bifenthrin (Figure 17.4) provided an 81.5% reduction of mosquito entry and 91% reduction in mosquito biting in treated tents over a 10-day period in Northern Territory, Australia.[62]

The protection of humans from mosquitoes by applying insecticides inside and around dwellings and community areas has been used for many years. Indoor residual spraying with DDT was used to control malaria by reducing the longevity of mosquitoes in many communities during the 1970s.[63]

Permethrin and bifenthrin have also been applied to vegetation as barrier treatment against mosquitoes.[64,65] A comparative trial of both these pyrethroids in Kentucky in 2004 showed that application to low-lying vegetation did not properly target adult resting sites of *Culex*, but can reduce *Aedes* mosquitoes. The study showed no differences between the two pyrethroids and both provided increased protection against mosquitoes.[66] The use of insecticides, especially permethrin, has been shown to enhance the barrier effect of tents in preventing the entry and mosquito bites within and around treated tents. This method was first evaluated with the application of repellents such as deet on tent fabrics. Sholdt et al.[67] showed that mosquito bites were reduced in and near tents treated with the repellent deet. The treatment of the inner walls of tents with permethrin reduced the nuisance of mosquitoes and invasive pests[59] and provided good protection against vectors of malaria.[61]

Figure 17.3 Habitats created for *Aedes aegypti* eggs and larvae in tent folds.

Figure 17.4 Application of bifenthrin insecticide to the outside/inside of Australian military tents.

Qualls et al.[68] compared the large-scale treatment of a golf course with the chemical bifenthrin with an area that only used ultra-low volume (ULV) spray to control floodwater mosquitoes in Florida. Both methods significantly decreased mosquito populations, compared to a control, but the two treatments did not differ. The mosquito population in the golf course treated with bifenthrin decreased by 84% compared to a 52% decrease in an area where ULV was used. The authors showed that the service requests in the barrier-treated area was decreased compared to the ULV-treated area, and the overall cost savings were estimated to be $2700. The authors concluded that barrier spraying was an appropriate tool for control of mosquito populations.[68]

CONCLUSION

Personal protection is used by military personnel, mining personnel, and tourists. Protection of expatriate miners who fly into mines located in malarious areas and periodically return to their home location where the risk of disease is reduced or absent. This may be the case in Africa, Southeast Asia, and Australasia. In Australasia, there are many mines operating in Papua New Guinea and Irian Jaya, where workers are flown in and out of the mine site on a rotational basis. The mining companies need to maintain production and put an important emphasis on protection against vectors of diseases such as malaria and dengue. Many mining companies provide uniforms pretreated with permethrin and advocate the correct wearing of uniforms by staff in the mine site and local areas. Military groups are also encouraged to correctly wear protective clothing, whereas the use of topical repellents on exposed skin is advocated by most of the groups of people in this study.

The use of personal protection measures against vectors of diseases, especially in developing countries particularly in Africa and Asia, has focused on methods that are low cost and can be conducted by local communities. In the last decade, more funds have become available to pursue some of the innovative methods of protection, and progress to reduce the incidence of vector-borne diseases, especially malaria, were made. The vector control workers in these countries have contributed knowledge and expertise in using and developing new methods.

This chapter discussed the use of innovative personal protection methods that reduce vector and nuisance biting and disease transmission among people in varied locations around the world. Despite a variety of methods, mosquitoes still cause nuisance and transmit diseases, and it is believed that the wider use of the methods described here and those under development will further decrease the burden of vector-borne diseases in the world. It is also sobering to note that personal protection products are sometimes subject to "false" advertising, which states untested and sometimes unrealistic outcomes for success.[69]

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CHAPTER 18

Best Practices for Use of Personal Protection Products

Daniel Strickman

CONTENTS

INTRODUCTION

This volume reviews many details about tools available to the individual for protection from arthropod bites. New products come on the market constantly and some represent real improvements to old capabilities. Over the years, a few products have been innovative additions to the kinds of protection available, for example, pretreated bed nets[1] and clothing.[2] More recently, effective spatial repellents that can be carried by an individual have provided protection in a way that ineffective products attempted to achieve for many years.[3]

In this chapter, we propose some principles and tactics for personal protection that attempt to assemble all the tools available into a system appropriate for different situations. The strategy consists of the following general steps to minimize bites: identify, suppress, avoid, block, repel, and respond. Although a person might take all of these steps for protection all the time, as sometimes implicitly recommended,[4] it is more reasonable to match the kinds of protection to the needs of the location, season, and time.[5]

IDENTIFY

Identifying the problem is a real challenge for the individual. Most people are not concerned with arthropods until they perceive a problem; therefore, their familiarity with different kinds of arthropods is very limited. It is not uncommon to meet people who mistake an aphid for a bed bug, a midge for a mosquito, or a stable fly for a tabanid. Guides to identification are common in the form of extension-style fact sheets, Internet species pages, comprehensive pictorial guides, and computer applications. The first inclination may be to match the insect seen with an image associated with a common name that comes to mind. This procedure is often helpful, but it is only likely to succeed when common language provides an entry point for the identification. For example, a person who thinks that a mosquito bit him or her can search on that word to find images with a good chance of confirming the identification of a real mosquito. On the other hand, commonly used terms such as gnat, sand fly, or sand flea are associated with many different insects. Less common terms may not even be familiar to the individual. From a professional standpoint, one might only expect a chance of accurate identification when the individual uses the most common terms such as tick, louse, bed bug, mosquito, or flea. Regionally, people may be familiar with a few more insects, such as black fly or biting midge. Entomologists are often willing to identify arthropods for the public, but between the difficulty of capturing the biting arthropod and the scarcity of entomologists, such identifications for the individual are probably rare.

Even less reliable than individual identification of arthropods is individual identification of bites. A person with any kind of lesion on the skin may suspect an arthropod as the cause, but conditions as varied as self-scarification to allergic reaction can be very similar in appearance to some kinds of bites. Even a professional will have difficulty identifying the cause of bites, though general features of some bites can be characteristic. For example, biting midges in the southeastern United States leave a pancake-round, flat welt with a small red petechial center. On the other hand, mosquito bites are notoriously variable and also change in appearance as the skin reaction progresses.[6] Nonetheless, the first sign of a problem for the individual is often a bite rather than an arthropod, which creates uncertainty over how to respond.

In special situations, the public is informed about the nature of an arthropod-borne disease threat. Those announcements typically include warnings about the vector arthropods, so that there is a good chance that the public at large will recognize the need to prevent certain kinds of bites. Perhaps one of the commonest examples is malaria. Travelers to malarious areas are usually warned about the disease and advised to use repellents as well as chemoprophylaxis (e.g., http://www.cdc.gov/malaria). Travelers with the most concern may try their best to make informed decisions about self-protection, choosing repellents, bed nets, or area repellent devices that will suit their individual needs. Another commonly communicated threat is Lyme disease (e.g., http://www.ct.gov/dph/cwp/view.asp?a = 3136&q = 395590), with associated messages about deer tick (*Ixodes scapularis*) identification, how to recognize the typical bull's-eye rash, and specific advice on avoiding tick bites.

Identification of the biting arthropod is important both to know the preventive measures necessary and to assess the risk of disease. Recognition of the species may be important in some cases, for example, deer ticks and Lyme disease, but more general identification will often be sufficient to determine how and when to take measures to prevent bites. Connecting the individual with identification of a specific, treatable condition is a big challenge for society, whether to fix a washing machine or to cure cancer. Appliances and medicine have their own dedicated cadres of professionals trained, positioned, and compensated to inform the individual. Entomology largely lacks such a system, so the majority of individuals are left on their own to determine the nature of their arthropod problem.

SUPPRESS

Personal protection is usually associated with the use of products and devices to stop bites, but one of the first steps an individual should take is to attempt to limit the source of the problem. Of course, such measures are only possible at fixed locations where sources of biting arthropods can be modified or eliminated. Home pest control is beyond the scope of this book, but certain measures are so important that they cannot be ignored as part of best practices for personal protection. General insecticide application will often provide relief from many different kinds of arthropods regardless of their sources, but certain problems can be eliminated, or nearly so, by removal of sources where the arthropod develops.

Water-filled containers are a common source of some kinds of mosquitoes. The proximity of those containers to the home result in a situation where the resident is most susceptible to the bites from mosquitoes produced on his or her own property. Use of repellents, bed nets, or other standard tools of personal protection may not make sense if the problem can be solved by eliminating, draining, or treating containers within the individual's control. Other kinds of mosquitoes that develop at the edge of ponds, streams, or in ground pools may present a problem more difficult for the individual to solve. Modification of those sites can reduce their productivity, but such measures may often be beyond the capabilities of an individual.

Several kinds of biting mites can infest homes and bite residents. Those infestations are associated with rodents or birds. Exclusion of rodents and birds may require extensive structural modification. Rodents can be particularly difficult to keep out, entering through small gaps under doors, around pipes, or in screens. Sanitation both indoors and outdoors can also help discourage rodents by denying them water and food sources. Once a home is sealed against rodents, measures can be taken to eliminate them by trapping or baiting. Mites may bite human residents more as the rodents disappear; however, the mites cannot complete development without the rodents.

Fleas, usually *Ctenocephalides felis*, are another problem often associated with animals in the home, but in this case the animals are pets. Modern systemic insecticides for dogs and cats have provided a tool that can solve many flea problems. Efficient and safe insecticides for treatment of carpets and furniture are also available, reducing the number of adult fleas and preventing development of immature stages. The human flea, *Pulex irritans*, lives in close association with people and their bedding. Those infestations can sometimes be eliminated with sanitation, but just as for bed bugs, professional help is likely to be necessary. In any case, elimination of these sorts of infestations makes a lot more sense than trying to prevent bites through personal protective measures.

AVOID

Biting arthropods are not equally distributed in either space or time. Avoiding them can be a very effective way of not getting bitten. Highly seasonal pests, such as the black gnat of the western United States (*Leptoconops* spp.), may be sufficient reason to avoid areas where they are abundant in the early spring.[7] People commonly avoid forested areas of the northeastern United States because of the risk of Lyme disease. The majority of certain species of mosquitoes bite during a couple of hours after sunset,[8] which should be encouragement to stay indoors during those times. Historically, tsetse flies (*Glossina* spp.) made entire areas of Africa virtually uninhabitable because of the threat from sleeping sickness.[9] Effective avoidance depends on good knowledge of the biting arthropods, which as pointed out earlier, is difficult to communicate to the public.

BLOCK

In some environments, a large part of being indoors is having walls, windows, roofs, and screens between the occupants and insects outside. Light construction from local materials in the tropics does not always provide this kind of protection. Under these circumstances, aspects of construction we take for granted may be absent, such as solid connections between roof and wall, tightly fitting doors, window screens, and even the presence of four walls. Any problem with flying, biting arthropods outdoors is likely to be the same indoors. When possible, a home that does not allow free access to insects is a good strategy for personal protection. The same principles apply to tents for temporary shelter. Keeping insects out involves practice as well as construction, for example, by keeping screens in good repair and discipline about shutting exterior and interior doors.

Bed nets are a good barrier when sleeping outdoors or indoors in a house that allows access to biting arthropods. Bed nets come in many forms, but there are several principles to consider when selecting one. First, the mesh size should be small enough to stop the insects but as large as possible to allow air flow. Probably the largest practical mesh for mosquitoes is nine openings per linear centimeter but stopping sand flies or biting midges requires much finer mesh. Second, the geometry of the net should minimize contact with the sleeping person inside so that bites are not received through the net. This means that the net, and bed within, are a little larger than usual and that the sides of the net do not slope inward directly over the sleeper. Box-shaped nets are often used, but they require more support than the common conical net suspended from one point directly above the bed. It is often necessary to use rolled towels, blankets, or even stuffed animals to hold a sloping net away. Finally, nets in daily use should be practical—colored to hide dust and dirt, easy to wash, and easy to put away each morning. Bed nets treated with pyrethroid insecticides have been distributed by the millions and they have made a big difference reducing malaria.[10]

Clothing is another important barrier against biting arthropods. Mosquitoes commonly bite through cloth that is tight against the skin. Tighter weaves and some fibers[11] make a better barrier. Wearing loose-fitting clothing and double layers also prevents bites. Permethrin-treated clothing also prevents many bites through cloth, though it does not protect adjacent skin. Loose-fitting suits of durable mesh are available for situations with very high biting pressure.

Tick bites can be reduced by proper wear of clothing. The main principle is to force a tick crawling up from the feet to stay on the outside of trousers. This can be done by blousing the trousers into boots or by pulling socks outside the bottom of the trousers. A shirt should always be tucked into the trousers to avoid ticks crawling from the belt line onto skin. Black flies also like to walk under clothing or hair before biting, which can be reduced by wearing long sleeves with tight cuffs and by wearing a hat. Black flies are more persistent than ticks and able to hover near a person even as he or she moves. A black fly that is discouraged by a tight cuff or hat may end up biting near the edge of that barrier, possibly having been dislodged repeatedly, but flying back to the host each time.

Ticks are the commonest biting arthropods that are commonly carried on clothing into the home. Other arthropods carried on clothing, including scabies mites (*Sarcoptes scabiei*), body lice (*Pediculus humanus humanus*), and sometimes bed bugs (*Cimex lectularius*), are usually treated as an infestation rather than as a target for personal protection. The majority of tick species cannot establish a reproducing colony indoors, but the individual ticks carried indoors eventually attach to one of the occupants. Checking clothing for loose ticks after a trip in the field can help. Washing or simply drying clothes at a hot setting kills any ticks that might be on them.[12]

REPEL

Repellents are available in two forms: area and topical repellents. The area repellents are usually based on the release of an active ingredient into the air with the intention of forming a cloud of

chemical that prevents the entry of flying, biting insects. The chemical might kill the pests, irritate them so that they move away from the hosts, or hide the odors that identify the potential hosts to the insects. Area repellents are popular because they are easy to use and they offer a solution to biting problems that seems to affect the pest without affecting the host. In fact, a person may be exposed to chemicals by inhalation. Because of their reliance on producing adequate concentrations of the active ingredient in a volume of air, the performance of even the most effective area repellents will vary depending on air movement.

The appeal of an area repellent has resulted in many products that have a convenient format, but very low efficacy. This creates a situation in which the consumer must exercise considerable care to get meaningful performance from a product. The kinds of products that work the best have a mechanism to actively disperse a pyrethroid active ingredient into the air. Mosquito coils are the most common device, vaporizing the insecticide just behind the burning tip. Other devices use butane-powered catalytic heaters or even a candle to heat a paper pad impregnated with pyrethroid. Most recently, a battery-powered fan disperses a volatile pyrethroid, metofluthrin, from a device that can be worn. All of these area repellent systems prevent approximately 70%–90% of mosquito bites as long as the air around the user is still. The U.S. Environmental Protection Agency (EPA) has only registered area repellent systems for outdoor use, but overseas common devices electrically heat a pad impregnated with pyrethroid or a liquid reservoir of the active ingredient for indoor dispersal. These devices are generally very effective because the chemical reaches a higher concentration in the fixed volume of an enclosed room.

Best practice for use of area repellents depends heavily on realistic expectations for the products. Outdoors, efficacy will be variable so that the user is in the position of being pleased with performance when there are no bites but displeased when there are. Clearly, such performance is not a sound basis for disease prevention, as shown by meta-analysis of mosquito coils showing no measurable effect on malaria prevention.[13] In contrast, area repellent systems can significantly reduce the annoyance from biting insects when conditions are right. The other aspect of best practice for area repellent systems is safety. There is evidence that smoke from mosquito coils causes health problems similar to secondhand cigarette smoke.[14] Devices that use other sources of heat or a fan to disperse a pyrethroid only expose the user to the active ingredient itself. Used according to label directions, these exposure levels should be well below toxicological thresholds. Indoor use of area repellents is more questionable from a safety standpoint. On the one hand, they achieve reliable effectiveness by maintaining constant concentration of a pyrethroid in the air. On the other hand, the user may be exposed daily for hours to those higher concentrations. Indoor devices have been used by millions of people without reported harm; however, a thorough investigation of their safety would increase confidence in their use. For the traveler, a few days of exposure is almost certainly safe, though there should be confidence that the chemical in the device is not toxic.

Topical repellents are sold in dozens of different products containing a variety of active ingredients.[5] The repellents that use active ingredients of botanical origin may provide reasonable protection or little protection, but they usually do not last as long as the industry standard ingredients. Some botanical ingredients can also cause skin irritation through sensitization, despite their natural origin. There are botanical topical repellents that are highly effective, especially those containing *para*-menthane-3,8-diol (PMD; often labeled as oil of lemon eucalyptus). Recent development of what appears to be good repellents from mixtures of botanical active ingredients and the discovery of entirely new compounds gives hope that botanicals will become much more reliable in the future.

The standard active ingredients include deet, picaridin, IR3535, PMD, and DEPA (in India). Although all of them provide almost complete protection from a wide variety of biting arthropods, selection of active ingredient can make a difference for the consumer. Some very general guidance based on the advantages of each compound might be to use deet or DEPA when cost is an issue, to use PMD to protect against *Anopheles* vectors of malaria,[15] to use IR3535 against ticks[16] or for maximum safety,[17] and to use picaridin for good application characteristics and excellent efficacy.

Some general recommendations on the use of topical repellents apply to all products. A registered product has been tested for safety by an authority with some level of legitimacy and should receive more confidence than an unregistered product. Directions on a label, particularly for U.S. EPA-registered products, represent a careful synthesis of what will work and what is safe. Some of the directions are about safety, but applying the correct amount is about efficacy. It is easy to apply too little repellent and then experience less protection. In general, it is wise to wash repellent off skin and clothing when there is no longer a threat from biting arthropods. Children should not be allowed to apply repellent by themselves, as they will be less able to avoid getting the materials in eyes, nostrils, and ears. Another measure to avoid getting repellent where it is not wanted is to either use a product that does not get on the palms of the hands during application or to wipe the repellent off the palms after application. Repellent should never be applied to damaged, sunburned, or broken skin. Repellents for animals should never be used on people, and human repellents should never be applied to animals. Surprisingly, some repellents that are perfectly tolerated by humans are damaging to dogs, cats, or other animals.

Finding the correct topical repellent for an individual depends on the goal of the protection and the situation. Complete reliance on repellents for protection from vector-borne pathogens is probably not a good idea, but as discussed elsewhere in this volume, repellents can be a significant supplement to other protective measures. More commonly, a person is simply trying to avoid the discomfort of bites. Under those circumstances, the choice of products depends on the satisfaction with the results and the acceptability of the repellent. Individual preference for skin feel, odor, and ease of application is important. If a person is not satisfied with the application characteristics of the product, then the repellent may remain in the bottle and provide no protection at all.

RESPOND

Response to potential illness resulting from infection by arthropod-borne pathogens is as important as attempts to prevent the exposure. Individuals who inform themselves about the threat of disease associated with arthropods in their area should learn the simple, initial symptoms of those diseases. Unusual rash, fever up to 2 weeks after exposure to the bites, or even a feeling of illness out of the ordinary should be the occasion to seek medical attention and to point out to the physician that bites have occurred. Bacterial pathogens such as those that cause typhus, spotted fever, Lyme disease, and the ehrlichioses can be treated with antibiotics. Malaria usually responds well to treatment with special drugs for that purpose. Delay in treatment can result in much more serious consequences. Viral infections such as dengue, West Nile, or yellow fever can only be addressed with supportive therapy, but in some cases, that therapy can make the difference between life and death.

CONCLUSION

One of the advantages of personal protection is that the individual can in theory apply exactly the right solution for his or her own situation. The challenge with this approach is that most people are not generally informed about the kinds of biting arthropods or about the range of effective products available. Ideally, people would take a tiered approach to protection that intelligently integrates the use of information and tools. That approach starts with identification of the problem and significance of any health threat followed by suppression of potential sources of the pests within the individual's own control. When traveling or when affected by arthropods outside of one's own property, avoiding the areas and times when bites occur can help solve the problem. Physical barriers

can also help, and properly used repellents offer considerable protection. Finally, a person exposed to disease from pathogen-carrying arthropods can avoid some serious health consequences by being aware of initial symptoms and by seeking prompt medical attention.

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CHAPTER 19

Commercially Available Insect Repellents and Criteria for Their Use

Rui-De Xue, Gunter C. Muller, and Jonathan F. Day

CONTENTS

BACKGROUND

Numerous insect repellents under a variety of brand names are currently commercially available to consumers throughout the world. The selection of a repellent for use by a consumer depends on the pest and the preferred application method of the repellent. Generally, insect repellents fall into the following categories based on their intended use and method of application:

- Topical chemical repellents that are applied directly to skin.
- Spatial or area repellents that are applied between the human/animal and the immediate source of pest/vector arthropods. Usually they burn/heat or emit chemicals such as essential plant oils and insecticides.
- Plants, such as the citrosa-scented geranium (*Pelargonium* sp.), that are purported to repel mosquitoes from their immediate vicinity.
- Electronic repellers that use battery-powered ultrasonic or electromagnetic waves that supposedly repel mosquitoes, ticks, fleas, and cockroaches.
- Insecticide-impregnated fabrics or clothing, such as permethrin-treated clothes, bed nets, and curtains.
- Systemic formulations, such as vitamin B1, vitamin B12, garlic, brewer's yeast, and other plant-based chemicals, that have been reported to repel mosquitoes when taken orally.

Among the six repellent categories described, topical repellents are the most widely used. A majority of these contain deet (*N,N*-diethyl-*m*-toluamide) as the major active ingredient, although there are insect repellent products that contain other synthetic chemicals such as picaridin as their active ingredient. In addition, many commercial insect repellents contain distilled essential plant oils such as oil of lemon eucalyptus as active ingredients.

Pre-Deet Period

The use of insect repellents for protection from mosquito bites and other blood-sucking arthropods has a long history. Before World War II, the active ingredients in the few available insect repellent products were mostly natural plant oils such as oil of citronella. Oil of citronella, distilled from *Cymbopogon nardus* (citronella grass), has been used as a topically applied mosquito repellent since 1882. In outdoor situations, citronella candles are commonly burned, with limited efficacy to repel mosquitoes and other biting insects around porches, decks, and picnic areas. The repellent DMP (dimethyl phthalate) was discovered in 1929, indalone was patented in 1937, and Rutgers 612 (ethyl hexanediol) became available in 1939.[1] With the exception of oil of citronella, not many products that include one of these active ingredients are presently commercially available.

Deet-Based Products

Deet was discovered by McCabe et al.[2] and published in 1954 at the U.S. Department of Agriculture's (USDA) laboratory in Beltsville, Maryland; its biological activity was ascertained at the USDA laboratory in Orlando, Florida. Deet was formulated as an insect repellent in 1946 by the U.S. Army and was registered as a commercial insect repellent in the United States in 1957. Today, deet is the active ingredient in a variety of insect repellent products. There are approximately 230 deet-based products manufactured commercially under more than 70 brand names currently registered with the U.S. Environmental Protection Agency (EPA). These products are available to the public in a variety of formulations, including liquids, lotions, sprays, creams, and impregnated fabrics. The products registered for topical application to human skin contain 4%–100% deet as the active ingredient. Approximately 35% of the U.S. population is estimated to use deet every year.[3]

Non-Deet Products

There are several non-deet or deet-free products currently available to consumers. The MGK Repellent 326 (di-*n*-propyl isocinchomeronate) was registered by the USDA in 1957 as an insect repellent for livestock; however, this repellent has always been mixed with deet when formulated for human use. Presently, approximately 15,000–20,000 lbs of MGK Repellent 326 are sold annually. The non-deet repellent picaridin [1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester] has recently been added to the list of repellents recommended by the U.S. Centers for Disease Control and Prevention (CDC). The repellent IR 3535 [3-(*N*-butyl-*N*-acetyl)-aminopropionic acid, ethyl ester] was registered as an active ingredient and licensed for sale as an insect repellent in 1999. One end product, Avon's Skin-So-Soft Bug Guard Plus IR3535 Expedition, containing this active ingredient, is currently available in the U.S. market. In India, DEPA (*N,N*-diethylphenyl acramide) was synthesized by Kalyanasundaram (1982)[4] and is currently licensed for use in that country.[5]

Botanicals

Plant oils used as active ingredients in insect repellents are usually essential oils that are distilled from various parts of plants including leaves, flowers, fruits, stems, and wood. Essential oils have been used for a long time to repel and/or kill insects. They can be applied as liquid sprays and aerosols, creams, gels, crystals, pellets, and by impregnating the oils into materials that are burned to produce smoke. There are currently about 30 plant-oil-based repellent products registered and marketed in the United States. Most of the botanical repellents contain oil of citronella, eucalyptus, geraniol, soybean, or cedarwood as the active ingredient. Oil of citronella is registered as an insect repellent and/or feeding deterrent from feeding on plants, and as a repellent for use on animals. Two citronella oil-based products are currently marketed: "Ceylon type" (derived from *Cymbopogon nardus*) and "Java type" (derived from *Cymbopogon winterianus*). The CDC (May 2005) added oil of lemon eucalyptus (containing *p*-menthane-3,8-diol or PMD as the dominant active component) and picaridin to deet on its list of recommended repellents for human use. Oil of lemon eucalyptus is a plant-based mosquito repellent that provides a protection time from mosquito bites similar to that of low-concentration deet products.[6] Oil of lemon eucalyptus is available in a variety of formulations under different brand names throughout the United States. These include Repel Lemon Eucalyptus (containing the botanical extract) and OFF!® Botanicals Insect Repellent (containing synthetic PMD).

AVAILABLE COMMERCIAL INSECT REPELLENT PRODUCTS

Deet-Based Products

Some of the major deet-containing products as of 2012 are listed in Table 19.1:

- Amway's Hour Guard (Amway, Ada City, Minnesota): A spray and cream formulation containing 31.58% deet, 1.75% other isomers, and 66.67% inert ingredients.
- Ben's Tick & Insect Repellent (Botach Tactical, Los Angeles, California): A lotion containing 23.75% deet, 1.25% other isomers, 5% *N*-octyl bicycloheptene dicarboximide (MGK 264), 2.5% di-*n*-propyl isocinchomeronate (MGK 326), and 67.5% inert ingredients.
- BugOut (Products, Houston, Texas): A spray formulation containing 14.25% deet, 0.75% other isomers, and 85% inert ingredients.
- Cutter Skinsations (United Industries, St. Louis, Missouri): A spray formulation containing 6.65% deet, 0.35% other isomers, and 93% inert ingredients; and another aerosol spray formulation

Table 19.1 Major Deet-Based Insect Repellents Currently Available to Consumers

Product Brand Name	Formulation	Deet (vol:vol) Concentration (%)
Amway Hour Guard	Cream	32
Ben's Back Yard	Lotion or pump spray	24
Ben's Max	Lotion or pump spray	95
Ben's Wilderness	Aerosol	27
Ben's Wilderness	Pump spray	30
BugOut	Spray	14
Bugg Spray	Spray	25
Cutter Just for Kids	Spray	5
Cutter Skinsation	Spray	7
Cutter All Family	Aerosol	10
Cutter	Lotion with sunscreen (SPF 15)	10
Cutter Backwoods	Spray or aerosol	23
Cutter Outdoorsman	Aerosol	30
Eckerd	Lotion	14
Everglades	Spray	29
Everglades Max	Spray	95
Muskol	Lotion	29
Muskol Ultra	Spray	38
OFF! For Kids	Spray	5
OFF! Skintastic Unscented	Spray or lotion	7
OFF! Skintastic Fresh Scent	Lotion	7.5
OFF! Unscented	Aerosol	15
OFF! Deep Woods Unscented	Aerosol	30
OFF! Deep Woods Sportsmen	Aerosol	30
OFF! Deep Woods	Spray	100
OFF! Deep Woods Sportsmen	Spray	100
Repel Soft Scented	Gel	7
Repel for Kids	Lotion	10
Repel Family Formula	Lotion	10
Repel Family Formula	Spray	18
Repel Family Formula	Aerosol	23
Repel Family Formula	Spray	100
Repel Soft Scented	Spray	18
Repel Sportsmen Formula	Spray	18
Repel Sportsmen Formula	Lotion	20
Repel Sportsmen Formula	Aerosol	29
Repel Sportsmen Formula	Aerosol	40
Repel Hunter's Pump	Spray	55
Repel 100	Spray	100
Sawyer	Controlled release	19

containing 9.5% deet, 0.5% other isomers, and 90% inert ingredients. There are several other products available with a variety of concentrations of deet under this brand name.

- Eckerd (Eckerd Drug, Clearwater, Florida): A lotion containing 14.25% deet and 85.75% other isomers and inert ingredients.
- Muskol (Schering-Plough Health Care Products, Mississauga, Ontario, Canada): A lotion containing 28.5% deet, 1.5% other isomers, and 70% inert ingredients. Muskol Ultra is a spray formulation containing 38% deet, 2% other isomers, and 60% inert ingredients.

- OFF! (S. C. Johnson & Son, Racine, Wisconsin): There are about eight products in the market under this brand name containing deet in different concentrations ranging from 5% to 100%. These products are available as sprays, aerosols, and lotions.
- Repel (Wisconsin Pharmacol, Jackson, Wisconsin): There are more than 10 products in spray, aerosol, lotion, and gel formulations on the market under this brand name containing 7%–100% deet. It is also called Insect Block.
- Sawyer (Coulston Products, Easton, Pennsylvania): It is a controlled-release lotion formulation containing 19% deet.

Major Non-Deet Chemical Products

- Avon Skin-So-Soft Bug Guard Plus IR 3535 (AVON Products, New York, New York): A cream formulation containing 7.5% IR 3535 as active ingredient and 92.5% other ingredients. There is also a 20% formulation marketed as the "Expedition" form of the product.
- DEPA is registered in India as mosquito and cockroach repellent. Several reports have shown the efficacy of DEPA to be the same as deet. This product is recommended as an alternate to deet in India. Manufacturer information is not available.
- Autan or Bayrepel: This product contains 10% KBR 3023 or picaridin. It is registered and marketed in Europe, Canada, and other countries (Bayer, Dublin, Ireland), and more recently (2005) in the United States. Cutter Advanced Picaridin Repel is now available. The active ingredient is currently registered by Lanxess (Leverkusen, Germany).

Botanical Products

Two types of botanical insect repellent products are available in the market. Most products are of the type of botanical insect repellents that do not have registration with governmental agencies. Some brands of products, such as Bygone Bugz and GonE! Neem Aura, can be found from Mother Earth and herb medicine stores. Manufacturers claim that these products are effective insect repellents, but most (70%) of the products do not have EPA registration under an exemption for ingredients "generally regarded as safe." Some of these products find an alternative legal recognition in the United States through trademark (which is only concerned with protection of the brand name itself), patents (presumably implying some level of efficacy), or the U.S. Food and Drug Administration (usually as a cosmetic and therefore with an implication of safety rather than approved efficacy).

A second type of botanical insect repellent includes those that have essential plant oils as their active ingredient. Some of these active ingredients are registered by the EPA. Among these are oil of citronella, soybean oil, and oil of lemon eucalyptus. Some of the common formulations include:

- Alfresco (Alfresco Ltd., London, United Kingdom): A British product in lotion formulation containing 14 ingredients and marketed in Europe. This product is not registered in the United States.
- All Sport (SPF-30) (All Sport, Ft. Lauderdale, Florida): This product is a spray formulation that is not registered by the EPA.
- Avon Skin-So-Soft (naturale) (AVON Products, New York): A lotion formulation containing oil of citronella. It is registered and marketed in the United States.
- Ballet (mosquito repellent) (Buyline, NBI, Kenya): This repellent is manufactured in Kenya and is marketed in African countries. It is not registered and marketed in the United States.
- Bio Schutz: A lotion formulation of natural oils produced in Germany and marketed in Europe. Manufacturer and other information are not available.
- Bite Blocker (Consep, Bend, Oregon): A lotion formulation containing 2% soybean oil and other natural oils.

- BugBand Insect Repellent (EES, Cartersville, Georgia): A lotion formulation containing 20% geraniol and that is not registered with the EPA.
- Bite Stop (World Promotions and Marketing, Port Orange, Florida): A spray formulation containing geraniol, citronella, and cedar oils.
- Bugg Spray (Bugg Products, LLC, Long Lake, Minnesota): A spray formulation containing primarily peppermint oil (0.05%). EPA-approved product.
- Buzz Away (Dirt Works, New Haven, Vermont): A spray formulation containing 5% oil of citronella.
- Bygone Bugz (Lakon Herbals, Montpelier, Vermont): A lotion formulation available in the market as natural product repellent but not registered by the EPA.
- GonE! (Aubrey Organics, Tampa, Florida): A lotion formulation not registered by the EPA.
- Mosquito and Insect Shield (Mosquito Solutions, Port Orange, Florida): A lotion formulation containing catnip oil but not registered by the EPA.
- MosquitoSafe (Naturale, Great Neck, New York): A lotion formulation containing 25% geraniol oil but not registered by the EPA.
- Natrapel (Tender, Littleton, New Hampshire): A spray formulation containing 10% oil of citronella, EPA-registered, and available in the United States.
- Neem Aura (Neem Aura Naturals, Alachua, Florida): A spray formulation that is available in the United States but does not have an EPA registration.
- OFF! Botanicals insect repellent (S. C. Johnson & Son, Racine, Wisconsin): A lotion formulation containing PMD, the synthetic form of the active ingredient in the distillate remaining following extraction of oil of lemon eucalyptus. It is registered in the United States.
- Quwenling: A lotion formulation marketed in China and in several Asian countries. Its active ingredient is the distillate remaining following extraction of oil of lemon eucalyptus from the leaves of *Corymbia citriodora*. The major active ingredient, PMD, was first discovered when this product was chemically analyzed by the USDA. Chinese product. Other information is not available.
- Repel Lemon Eucalyptus (Wisconsin Pharmacol, Jackson, Wisconsin): Available in spray and lotion formulations and contains 30% oil of lemon eucalyptus (actually, the distillate remaining following extraction of the oil). Registered and available in the United States.
- SunSwat (Kiss My Face Corporation, Gardiner, New York): A spray formulation mixed with sunscreen active ingredients. It is marketed in the United States but does not have an EPA registration.
- Swamp Buddy Bug Chaser (TRU Ventures, Charlton County, Georgia): A spray formulation containing natural plant oils including 12% lemon grass, peppermint, eucalyptus, and other plant fragrances. This product is marketed on the Internet and does not have an EPA registration.

Spatial Repellents and Other Products

Permethrin is a pyrethroid insecticide that has minor repellent properties. It is commonly applied to clothing as a 0.05% permethrin solution. Repel Permanone is marketed as a repellent and insecticide that can be applied to clothing as a spray. Repel Camp Fogger (Wisconsin Pharmacol Co. Inc., Jackson, Wisconsin) and Cutter Backyard Insect Repellent (United Industries, St. Louis, Missouri) are aerosol formulations of 2.5% permethrin, which serve primarily as barrier-insecticide treatments with minor repellent properties.

Spatial or area repellents are designed to repel and kill biting arthropods in relatively small, semienclosed areas. These products are generally formulated as candles, coils, or products with some type (often butane) of catalytic converter to burn and passively dispense the active ingredient. These products often contain either essential oils, such as oil of citronella, or allethrin as the active ingredient. Commonly available products include Cutter Area Repellent Holiday Bucket Candle and Trip Wick citronella candle (United Industries), OFF! PowerPad lamp and lantern, OFF! Yard and Deck Area Repellent II, OFF! Mosquito Coil III, OFF! citronella

candle, OFF! Clip-on™ mosquito repellent[12] (S. C. Johnson & Son, Racine, Wisconsin), and ThermaCELL® mosquito repellent (The Schawbel Corporation, Bedford, Massachusetts).

USE OF INSECT REPELLENTS AGAINST MOSQUITOES, TICKS, FLEAS, AND OTHER BITING ARTHROPODS

Deet-Based Products

The deet-based insect repellents are the most widely used insect repellents to repel mosquitoes (Culicidae), biting midges (*Culicoides* and *Leptoconops* spp.), sand flies (Psychodidae: Phlebotominae), stable flies (*Stomoxys calcitrans*), black flies (Simuliidae), ticks (Argasidae and Ixodidae), chiggers (Trombiculidae), and fleas (Siphonaptera), but they do not provide satisfactory repellency for deer and horse flies (Tabanidae).[7] The branded deet-based products distributed by Amway, Ben's Insect Repellents, Cutter's, Muskol, OFF!, Repel, and Sawyer's insect repellents claim to repel mosquitoes, black flies, biting midges, stable flies, fleas, chiggers, and ticks for several hours.[7]

Non-Deet Products and Botanicals

The non-deet insect repellents as well as botanical-based insect repellent products registered by the EPA are usually used against mosquitoes and ticks. Products that contain IR 3535 provide relatively short protection times against mosquitoes, although other tests have shown that IR 3535 is roughly equivalent to deet in efficacy. Products containing picaridin,[6] PMD, and Bite Blocker (soybean oil) provide protection times equivalent to those provided by low concentrations of deet.[8] Products that have botanicals as active ingredients, such as oil of citronella or geraniol oil, usually have short-term repellent efficacy against several species of mosquitoes.[9] For example, MosquitoSafe, containing 25% geraniol oil, provides 2–3 hours of protection from mosquito bites in the field,[9] Bite Blocker provides 6–8 hours of protection against mosquitoes and ticks, and Repel Lemon Eucalyptus provides 6 hours of protection from mosquitoes and ticks.[6]

Spatial Repellent and Other Products

Insecticide repellent products containing permethrin as the active ingredient are usually used to spray and impregnate clothing for protection from mosquitoes, ticks, and other biting arthropods. Some products containing permethrin as an active ingredient are marketed as barrier treatments for protection from fleas, ticks, and mosquitoes. These barrier treatments generally provide residual insecticide protection for several days to several months, depending on the product. It is doubtful whether the use of these products is justified, considering their relatively low efficacy and the indiscriminate application of insecticide in the environment. Some of the common formulations are described in this section.

OFF! Clip-on mosquito repellent and ThermaCELL mosquito repellent as spatial repellents against mosquitoes provided significant protection.[10] Botanical repellents are highly volatile, so topical forms require frequent reapplication to provide protection. To overcome this, essential oils are being used as spatial repellents that emit active ingredient into the air by volatilization with heat, aerosol, or diffusion by air current. The efficacies of these products including candles, area, and personal diffusers seem to depend not just on the active ingredient but also on the method of emission, airflow through the area being protected, distance between product and user, and type of biting insect being repelled.

Candles impregnated with citronella, linalool, or geraniol (each 5%) are repellent against mosquitoes and, to a lesser extent, sand flies, when used outdoors with minimal airflow.[11] When placed

1.0 m apart, citronella candles reduced CDC trap catches of mosquitoes by 35.4% and sand flies by 15.4%, linalool reduced mosquitoes by 64.9% and sand flies by 48.5%, and geraniol reduced mosquitoes by 81.5% and sand flies by 69.8%. By increasing the distance to 2 m and 3 m, the repellency dropped significantly. In human landing catch studies indoors with little or no airflow, candles were still repellent at 2 m. The 5% citronella candles reduced mosquito and sand fly biting attempts by 29.0% and 24.7%, respectively, linalool by 71.1% and 55.2%, respectively, and geraniol by 85.4% and 79.7%, respectively.[12] Candles with geraniol were about twice as repellent as those with linalool and were about five times as repellent as citronella candles.

Several essential oil diffusers are commercially available in a tabletop form or a clip-on form that attaches to clothing. To date, only a few have been tested for efficacy. The Coleman Mosquito Deleto model 2950-602 is a small tabletop unit that heats 20 g cartridges, which can contain citronella, linalool, or geraniol for continuous release. Outdoors, citronella diffusers placed 20 feet from traps, reduced mosquitoes caught by 22%, linalool by 58%, and geraniol by 75%. Indoors, citronella diffusers reduced biting attempts by 68%, linalool by 93%, and geraniol by 97%.[13]

The Terminix ALLCLEAR Mosquito Mister (Lantern Edition) is another outdoor tabletop/hanging unit that disperses aerosolized aqueous geraniol (0.3%) emulsion in timed-release intervals of 5.0, 7.5, and 10.0 minutes. The degree of protection provided to human volunteers correlated well with the distance from the subject and the time interval of releases.[14] The 5-minute time interval mode reduced overall biting pressure by more than 90% at 9 ft. (2.74 m) and 18 ft. (5.49 m). Reduction of biting pressure in the 7.5-minute mode was still well over 80% and even in the 10-minute mode, overall protection was slightly above 80% at a distance of 9 ft. The lowest but still reasonable protection level was observed in the 10-minute mode, at the periphery of the area the unit claims to protect (300 ft.^{2}), with a biting pressure reduction of approximately two-thirds.

A human-landing catch comparison of four spatial repellents demonstrated clear differences between vaporization methods and choice of active ingredients used.[15] Products tested include the ThermaCELL Patio Lantern (a mat containing 21.97% d-*cis/trans* allethrin), OFF!®PowerPad lamp (a pad containing 21.97% d-*cis/trans* allethrin), Terminix ALLCLEAR Tabletop Mosquito Repeller (pad containing a mix of cinnamon oil 9%, eugenol 11%, geranium oil 17.6%, peppermint 4.4%, and lemongrass oil 2.2%), and Citronella Bucket Tabletop (a candle containing 0.5% citronella oil). Three of the four repelling products significantly decreased biting pressure on the volunteers at all distances tested, with the Citronella Bucket Tabletop being the only exception. Best results were obtained with the ThermaCELL Patio Lantern with a 96.1%, 89.9%, and 76.66% reduction at a distance of 1.0, 2.5, and 3.33 m, respectively, followed by OFF! PowerPad lamp (91.6%, 83.1%, and 64.3%, respectively) and the Terminix ALLCLEAR Tabletop Mosquito Repeller (90.4%, 77.1%, and 55.2%, respectively).

Clip-on diffusers, which attach to a person's clothing, offer continuous release of the active ingredient at a very short distance between unit and user. The OFF! Clip-on mosquito repellent (metofluthrin 31.2%) and the Terminix ALLCLEAR Sidekick Mosquito Repeller (cinnamon oil 10.5%, eugenol 13%, geranium oil 21%, peppermint 5.3%, and lemongrass oil 2.6%) offered superior protection compared to other personal repellents such as citronella-impregnated wristbands or stickers.[16] In this case, the essential oil–equipped unit performed nearly as well as the metofluthrin unit; biting was reduced by the OFF! Clip-on and the Terminix ALLCLEAR by 92.83% and 87.55%, respectively, against *Aedes albopictus*, and by 97.22% and 94.14%, respectively, against *Culex pipiens*. Super Band, Pic Wristbands, Sonic Insect Repeller, Mosquito Guard Patch, and Mosquito Patch-Vitamin B1 demonstrated poor repellency with mosquitoes freely landing on the products.

These studies suggest that natural ingredients in spatial repellents provide reasonable protection to people provided they are well volatilized, that they create a plume of repellent that surrounds a person, and that the active ingredient plume remains relatively undisturbed by airflow. Consumers should read product labels and take note of important product features such as distance from product, hours of use, and safety, such as warnings on inhalation of vapors.

APPLICATION RATE AND EFFICACY

Deet-Based Products

The higher the concentration of deet in a repellent, the longer the protection time.[17] In general, products that contain 5%–7% deet provide approximately 90 minutes of complete protection with a single application, whereas those containing 30% deet provide approximately 6 hours of complete protection with one application.[8] Products that contain more than 30% deet provide nearly 10 hours of protection from mosquitoes, stable flies, sand flies, fleas, and ticks.[7] Ultrathon (29%–33% deet) provides 12-hour protection from mosquitoes and ticks. Protection times from bites of *Aedes albopictus* provided by selected deet-based products under laboratory conditions are summarized in Table 19.2.

Non-Deet Repellents and Botanical Products

Under laboratory conditions, picaridin (7%) in a spray formulation provided protection from mosquito bites for nearly 6 hours, whereas IR 3535 (7.5% active ingredient) gave approximately 2 hours of protection from mosquito bites.[6,18] Bite Blocker, containing primarily 2% soybean oil, yielded longer protection (6–7 hours) from mosquitoes and ticks.[6] Products containing more than 25% geraniol oils provided less than 3 hours of protection,[6] and lemon eucalyptus oil gave 7–8 hours of protection.[6] Several non-deet and botanical products containing low concentrations of geraniol oil and oil of citronella as the active ingredient did not result in appreciable protection time from bites of *Aedes albopictus* (Table 19.3).[6]

Spatial Repellent and Other Products

Insecticide repellent products containing permethrin are used for outdoor and indoor barrier treatments, treatment of clothing, and treatment of bed nets and curtains.[19] Individual treatments last for several days to several months depending on the product used and environmental conditions, especially the amount of sunlight on the treated area. Products that contain oil of citronella, or insecticides that are burned to produce smoke for repelling mosquitoes, are used once and then replaced.

Table 19.2 Selected Deet-Based Insect Repellent Products and Their Efficacy against *Aedes albopictus* Tested in the Laboratory

Product	Formulation	Deet (%)	Protection (Hours)
Amway Hour Guard	Cream	31.58	13.2
Ben's	Lotion	23.75	7.5
BugOut	Spray	14.25	7.3
Cutter	Spray	6.65	5
Cutter	Spray	9.5	5.8
Eckerd	Lotion	14.25	6.8
Muskol	Lotion	28.5	6.7
Muskol	Ultra spray	38	8
OFF!	Spray	6.65	5
OFF!	Sunscreen cream	9.5	3.8
OFF!	Spray	14.25	7.2
OFF!	Spray	23.8	8.5
OFF!	Lotion	95	12.7
Repel	Spray	14.25	7.7
Sawyer	Controlled release	19	7.3

Table 19.3 Selected Non-Deet-Based Insect Repellent and Botanical Products against *Aedes albopictus* Tested in the Laboratory

Name of Product	Formulation	Active Ingredient	Protection (Hours)
EPA-registered products			
Picaridin	Spray	1-Piperidinecarboxylic acid	5.7
Avon Bug Guard	Cream	IR 3535 (7%)	1.8
MosquitoSafe	Lotion	Geraniol oil (25%)	2.8
Natrapel	Spray	Citronella (10%)	1.3
BiteBlocker	Lotion	Soybean oil (2%) and others	5.5
Repel	Lotion	Lemon eucalyptus (Quwenling)	7.8
Avon Bug Guard Natural	Lotion	Citronella oil (0.1%)	0.5
Not registered by EPA			
SunSwatspray	Lotion	Sunscreen materials and natural oils	0.2
Alfresco	Lotion	Natural products, United Kingdom	1.2
Ballet	Lotion	Natural product, Kenya	0.5
Bygone	Lotion	Peppermint oil	0.2
Bio-Schutz	Lotion	Natural product, Germany	6
GonE	Spray	Eucalyptus oil	0
Neem Aura	Spray	Neem leaf extract	0.2
Quwenling	Lotion	Lemon eucalyptus distillate	5.5
Swarm Buddy	Spray	Natural oil	0.5

FORMULATIONS AND CRITERIA FOR USE

Formulations

Commercial insect repellents are available in multiple formulations, including aerosol and pump sprays, lotions, creams, gels, controlled-release formulations, camouflage face paint, clothing treatments, and towelettes. EPA regulations require that the concentration of the active ingredients should be clearly disclosed as part of the product label. A variety of insect repellent products in many different formulations can be purchased in the United States from drug stores, supermarkets, sporting goods stores, and online outlets. Irrespective of the nature of the formulation, products that contain 10%–30% deet have been proven to be effective against mosquitoes and ticks.[7]

Slow-released insect repellent formulations have recently been developed and marketed. In these formulations, a lower concentration of the active ingredient is used without sacrificing the duration of protection. The U.S. military used a polymer-based cream containing 34% deet as active ingredient. This product, known commercially as Ultrathon, has been shown to be as effective as 75% deet in alcohol, providing up to 12 hours of protection against mosquito bites. Ultrathon aerosol spray (25% deet) is also available. Sawyer produces a controlled-release 20% deet lotion that traps the deet and releases it slowly on the skin surface. This formulation provides repellency lasting for approximately 5 hours, equivalent in time duration to that given by 50% deet preparations.[8]

Selection Criteria

As a general rule, products containing higher concentrations of deet provide longer lasting complete protection from insect bites. Mathematical models show that the effectiveness and persistence

of repellents in terms of protection is proportional to the level of dose (i.e., concentration of the active ingredient in the product).[17] Generally, high deet concentration products are best utilized in situations with relatively high density of insects (e.g., hunting or fishing) where repeated applications of an insect repellent may become difficult, and in areas where environmental conditions such as high temperature, humidity, or rainfall result in the rapid loss of repellent from the skin surface. Higher concentration deet-based products may also be selected for travel through areas where there is a high risk of insect-transmitted pathogens. Products consisting of technical deet (nearly 100% active ingredient) can be convenient because a small package can contain many doses of the repellent, if used carefully.

PRECAUTIONS

Consumers can reduce any risks associated with the use of insect repellents by reading, understanding, and following label instructions. These products should not be applied over cuts, wounds, or irritated skin. Also, overapplication (overdose) of the product to the skin or clothing should be avoided, and just enough of the product to cover the exposed skin or clothing should be used. Products containing more than 50% active ingredient should not be repeatedly applied to the skin over a short period. After returning indoors, treated skin should be washed with soap and water. Treated clothing should be washed before reuse. Products containing high concentration of an active ingredient may cause skin reactions. Aerosol and pump spray formulations should be used with great caution in enclosed areas. For face application, the product should be sprayed on hands first and then rubbed on face; direct spray on the face should be avoided.

Application of a product to the hands or near the eyes and mouth of children should be avoided because children may ingest the chemicals by putting their hands in their mouth. Children should not be allowed to handle or apply products by themselves. An adult should apply the repellent to his/her hands and then apply it to the child. When using a new product, a small quantity of the repellent should be tested first on a small area of the arm to check for any allergic skin reactions. If any such reaction occurs, product use should be immediately discontinued, and the skin receiving the repellent should be washed with soap and water. In the case of severe uncontrolled allergic reactions to a product, the local poison control center should be contacted. When visiting an emergency room for treatment to severe allergic reactions, the repellent container should be provided to the attending physician. In the case of children in the age range of 6–24 months, only one application of repellent per day is recommended by the CDC and the EPA. For older children (2–12 years old), the same product could be applied up to three times a day. All other individuals should read and follow label instructions on all repellent products.

CONCLUSIONS

At present there are numerous insect repellent products available commercially under a variety of brand names. Historically, the most efficacious repellents, in terms of complete protection time with a single application, have been those that contain deet as the active ingredient. However, in May of 2005, the CDC added repellents containing the active ingredients picaridin and oil of lemon eucalyptus (their intention was to include both synthetic PMD and preparations of Quwenling) to their list of recommended repellents for prevention of arthropod-borne pathogen transmission. This was done mainly to provide consumers with a selection of effective repellents, though the choice of active ingredients is somewhat controversial.[9] The EPA has registered other active ingredient compounds for insect repellents, such as IR 3535, citronella, and other plant oils (geraniol, cedarwood, and soybean). Once an insect repellent has been selected and purchased for application to human

skin, it is important to carefully read and follow the label directions. Arthropod repellents should be used when there is a threat of disease from arthropod-transmitted pathogens or when the nuisance biting exerted by populations of blood-feeding arthropods renders outdoor activity difficult or impossible. In general, the best advice is to avoid biting arthropods during their peak activity periods, usually from dusk to dawn. When it is difficult or impossible to avoid biting arthropods, cover as much skin as possible with a light-colored, loose-fitting, breathable fabric through which arthropods cannot bite. Finally, cover exposed skin with a personal arthropod repellent that has a reasonable (at least 60 minutes) complete protection time with a single application.

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CHAPTER 20

Alterations of Blood-Feeding Behavior and Repellent Response of Pathogen-Infected Biting Flies

Whitney A. Qualls, Rui-De Xue, John C. Beier, and Mustapha Debboun

CONTENTS

INTRODUCTION

The traditional view of interactions between pathogens and their arthropod vectors is that vectors become increasingly resistant to pathogens over time and parasites do not exhibit any deleterious effects when disseminating within their hosts.[1] This view assumes that if the host and pathogen do not coexist, a detrimental effect that hinders the parasite's fitness will be observed in the invertebrate host. However, the necessity of a pathogen to exploit its host efficiently and to gain access to new hosts has driven pathogen evolution that influences vector behavior. Studies have suggested that the pathogen manipulates the host to enhance their own transmission.[2–4] One such behavior that has been reported is pathogen manipulation of the vector's feeding behavior. Alterations of the feeding behavior leading to an increase in host contact can increase pathogen transmission.

The use of repellents is important in preventing arthropod-borne pathogen transmission. Investigation of pathogen-associated changes in arthropod behavior is critical to determine if repellents function in reducing or preventing medically important arthropods from biting or if such pathogen-associated changes can result in altered arthropod response, rendering repellents less effective. Few published studies are available for comparing the effects of pathogen infection and repellent efficacy in biting flies.[5–10]

IMPACT OF PATHOGEN INFECTIONS ON HOST-SEEKING AND BITING BEHAVIORS BY MOSQUITOES

There are a number of pathogens transmitted by biting flies including bacteria, viruses, protozoa, and filarial nematodes (Table 20.1). Investigations of pathogen–arthropod host interactions have resulted in numerous reports describing modifications of biting flies blood-feeding behaviors following pathogen infection. *Plasmodium*-infected mosquitoes,[11–13] *Leishmania*-infected sand flies,[14] *Trypanosoma*-infected tsetse flies,[15] and arbovirally infected mosquitoes[16–19] have all been shown to have higher host probing and/or biting rates than their uninfected counterparts (Table 20.2).

The blood-feeding behavior of Anopheline mosquitoes has been reported to be altered after *Plasmodium* infection. *Plasmodium* infections have been associated with an increase in probing behavior. Rossignol et al.[11] first reported that *Plasmodium gallinaceum* infection increased the probing time of infected female mosquitoes compared to uninfected ones. Wekesa et al.[12] collected *Anopheles gambiae* with natural infections of *P. falciparum* and found that 65% of infected females probed on a guinea pig at least three times compared to only 27% of uninfected females. The increase in probing of *Plasmodium*-infected mosquitoes is related to a decrease in an important enzyme in the salivary glands that facilitates the mosquito in locating a blood meal. Engorgement has also been demonstrated to be affected by *Plasmodium* infection. Koella et al.[13] reported that natural populations of *P. falciparum*-infected *Anopheles gambiae* were more likely to be fully engorged compared to uninfected individuals. The observed changes in these studies suggest that the manipulated changes in the mosquitoes' blood-feeding behavior allow the pathogen to spread more rapidly among human hosts by increasing the human–vector contact.

Both pathogen-infected sand flies and tsetse flies have been reported to have altered blood-feeding behaviors. *Leishmania* infection has been associated with an increase in sand fly refeeding

Table 20.1 Biting Flies and Pathogen Infections Affecting Human Health

Family	Common Name	Pathogen			
		Virus	Bacteria	Protozoa	Other
Culicidae	Mosquito	Dengue		Malaria	Filariasis
		West Nile			
		Eastern equine encephalitis			
		Rift valley fever			
		Yellow fever			
		St. Louis encephalitis			
		Japanese encephalitis			
		Venezuelan equine encephalitis			
		Chikungunya			
		La Crosse encephalitis			
Glossinidae	Tsetse fly			Trypanosomiasis	
Psychodidae	Sand fly	Sand fly fever	Bartonellosis	Leishmaniasis	
		Vesicular stomatitis			
Simuliidae	Black fly				Onchocerciasis

Table 20.2 Pathogen Mediate Blood-Feeding Behavior Changes in Biting Flies

		Response			
Vector	**Pathogen**	**Blood-Feeding (Time)**	**Probing (Time or Number)**	**Engorgement (Weight)**	**Refeeding (Number of Hosts)**
Anopheles gambiae	*Plasmodium falciparum*				
Anopheles gambiae	*Plasmodium falciparum*			Increase	Increase
Aedes aegypti	Dengue (serotype 3; *Flavivirus*)	Increase			
Aedes triseriatus	La Crosse (*Bunyavirus*)	Increase (time)		Decrease	
Aedes aegypti	Sindbis (*Flavivirus*)			Increase	
Lutzomyia longipalpis	*Leishmania mexicana*				Increase
Lutzomyia longipalpis	*Leishmania infantum*				Increase
Glossina morsitans morsitans	*Trypanosoma brucei*			No difference	

rate and promoting feeding on multiple hosts.[14] The feeding persistence and the initiation of multiple feedings is a direct manipulation by the metacyclic promastigote development stage of the *Leishmania* pathogen. This form of behavioral manipulation results in an enhanced parasite transmission. Tsetse flies salivary gland infection with *Trypanosoma brucei* is associated with prolonged feeding time.[15] Trypanosome-mediated modification of the tsetse salivary composition resulting in reduced antihemostatic potential and a hampered feeding performance has been suggested to lead to an increase in vector–host contact and parasite transmission.

Arbovirally infected mosquitoes demonstrated both increased probing and engorgement rates compared to uninfected mosquitoes. Both probing and feeding times were increased in *Aedes aegypti* mosquitoes infected with DENV-3 virus.[17] Infected *A. aegypti* required 10 minutes longer or more to complete feeding compared to noninfected individuals. Increased probing behavior and reduced rates of engorgement were observed in La Crosse virus-infected *Aedes triseriatus*.[16] Qualls et al.[8] demonstrated that Sindbis-infected *A. aegypti* mosquitoes have a decreased activation time, that is, the time it takes a mosquito to locate the host, and takes much longer to fully acquire a blood meal compared to uninfected control mosquitoes. Completion of the four stages of blood feeding took 1.3 and 1.5 times longer on days 7 and 14 post virus exposure, respectively, for mosquitoes with a Sindbis dissemination. La Crosse virus-infected *A. triseriatus* took significantly less blood and refed more often than their uninfected cohorts.[18] Overall, reductions in blood meal size followed by an increase in refeeding and an increase in probing could lead to enhanced virus transmission.

Studies have not yet identified the physiological mechanisms involved in pathogen-induced feeding alterations. However, researchers suggested that pathogen infection of organs and tissues that are known to control or influence activities associated with blood feeding might be associated with behavior changes. For example, dengue,[17,19] West Nile virus,[20] and Sindbis virus[21] have been shown to develop a significant infection of the nervous tissue. Early termination of feeding may be related to the neural disruption of the abdominal ganglia, which has receptors that signal the brain that enough blood has been imbibed.[22] These same stretch receptors also inhibit host-seeking behavior and may explain the increased refeeding activity in many arbovirally infected mosquitoes.[19,23]

IMPACT OF PATHOGEN INFECTION ON INSECT REPELLENTS BY BITING FLIES

With the pathogen-associated cytopathology of the nervous system, it has been suggested that the response of biting flies to repellents may be altered. Although this has been proposed, there is limited research evaluating the response of pathogen-infected arthropods to repellents. Overall, pathogen-infected host response to repellents varies depending on the pathogen and host evaluated (Table 20.3).[5–10]

A study evaluating the response of *P. falciparum*- and *P. berghei*-infected and uninfected *Anopheles stephensi* found no significant differences in the time to locate a host after exposure to *N,N*-diethyl-3-methylbenzamide (deet).[5] The response of *Plasmodium*-infected mosquitoes to other repellents has not been further evaluated to date.

Responses of arbovirally infected mosquitoes to repellents have been evaluated primarily using intrathoracically inoculated mosquitoes. Barnard et al.[6] reported *A. aegypti* infected with *Edhazardia aedis*, microsporidia, took about 56.8 minutes longer to bite a human hand treated with 15% deet compared to their uninfected cohorts. Frances et al.[7] found no altered response of *A. aegypti* and *Aedes albopictus* to deet after infection with any of the four dengue serotypes. These studies focused on behavior changes following intrathoracic inoculation of mosquitoes with arboviruses, which does not reflect the natural route of infection. Offering a mosquito an infectious blood meal, the virus enters the mosquito through the natural route and provides a more realistic representation of virus dissemination and replication in the mosquito. Thus, behavior changes observed after a natural route of infection and dissemination within the mosquito provide stronger evidence that those behavior changes could be observed in the field.

Using the natural route of infection, oral exposure to the virus, Qualls et al.[9] demonstrated that mosquitoes with a disseminated Sindbis infection after exposure to 30% deet had a reduced time to first bite compared to their uninfected cohorts. This investigation detected a decrease in sensitivity to the repellent deet in mosquitoes with a disseminated Sindbis infection. Compared to uninfected control mosquitoes, the time to first bite was reduced and both the first and fifth bites occurred 4 hours sooner in Sindbis-infected mosquitoes after exposure to deet. In other words, this decrease in time to first bite in mosquitoes with a Sindbis infection was not influenced by days post virus exposure and was not a single outlier as demonstrated by the time to fifth bite. In the presence of 30% deet solution, mosquitoes with disseminated Sindbis infection located a blood meal 3.2 times faster compared to their uninfected cohorts.

Qualls et al.[10] also demonstrated that a dissemination of Sindbis within *A. aegypti* was associated with a decrease in sensitivity to repellents containing deet [15% active ingredients (AIs)] and picaridin (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester; 15% AIs). Furthermore, Sindbis-infected mosquitoes took less time to locate and fully engorge on a blood

Table 20.3 Response of Pathogen-Infected Vectors to Repellents

Vector	Pathogen	Repellent (% Active Ingredient)	Time to First Bite
Anopheles stephensi	*Plasmodium falciparum*	Deet	No difference
Anopheles stephensi	*Plasmodium berghei*	Deet	No difference
Aedes aegypti	Dengue (serotypes 1,2,3,4)	Deet (30)	No difference
Aedes aegypti	*Edhazardia aedis* (microsporidia)	Deet (15)	Increase
Aedes aegypti	Sindbis (*Flavivirus*)	Deet (15)	Decrease
Aedes aegypti	Sindbis (*Flavivirus*)	Picaridin (15)	Decrease
Aedes aegypti	Sindbis (*Flavivirus*)	Oil of lemon eucalyptus (30%, 65% of *p*-menthane-3,8-diol)	No difference
Aedes aegypti	Sindbis (*Flavivirus*)	2-undecanone (7.75)	No difference

meal than uninfected mosquitoes when exposed to repellents containing the AIs deet, picaridin, and oil of lemon eucalyptus repellents (30% AIs; ~65% of *p*-menthane-3,8-diol). Mosquitoes with a disseminated Sindbis infection demonstrated a 46% and 37% reduction in time to first bite when exposed to deet and picaridin, respectively, compared to their uninfected counterparts. The repellents deet, picaridin, 2-undecanone (7.75% AIs), and oil of lemon eucalyptus did not inhibit Sindbis-infected *A. aegypti* from taking a blood meal during the expected protection time of the repellents. Sindbis virus is the prototype *Alphavirus* and an important entity to extrapolate to biosafety level-3 viruses. It should be noted that not all arboviruses replicate to the same degree in secondary tissues and this may result in why some behavior changes are reported for one arbovirus and not for another arbovirus.

Repellents function to either mask the chemical cues involved in locating a host or promote avoidance of the host, responses that are mediated by olfaction.[24] The decrease in time to first bite of Sindbis-infected mosquitoes after exposure to deet and picaridin may be because of damage or blockage of important proteins and neurons, involved in odor detection, by virus infection resulting in the odorant not being received or processed correctly. Responses of infected mosquitoes were not masked by the natural repellents, 2-undecanone and oil of lemon eucalyptus. Even though both 2-undecanone and oil of lemon eucalyptus elicit a repellent response, they may also activate the gustatory receptors, which could lead to a shift from host-seeking to sugar-feeding behavior.[25] These plant-based AIs may be attractive in the context of locating floral or extra-floral plant nectaries as the battle between suppression of odor receptor neurons and activation of gustatory receptor neurons begin.

CONCLUSION

The literature does suggest the pathogen manipulation of the vector occurs and has a direct influence on pathogen transmission. As seen in biting flies, pathogen manipulation generally results in an altered blood-feeding behavior that increases host–vector contact. Thus, increasing the probability of pathogen transmission.

When considering strategies that can be implemented to reduce public health concerns associated with arthropod-transmitted diseases, repellent use is one of the primary personal protection methods available. In fact, for diseases such as West Nile virus, the primary recommendation for protection as directed by the Centers for Disease Control and Prevention is for individuals to wear repellents. However, because of the deleterious effects of pathogens on the vector, olfactory processing may be influenced resulting in decreased sensitivity to certain repellents. Future research needs to identify how pathogen infection may affect repellent efficacy and those physiological mechanisms that result in a response that differs from uninfected vectors. Repellent development that specifically targets blocking odor receptors from being activated may be more advantageous for preventing pathogen transmission.

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CHAPTER 21

Insect Repellents for Other Potential Use

Rui-De Xue, Gunter C. Muller, and Mustapha Debboun

CONTENTS

INTRODUCTION

Essential oils and extracts from natural plants and synthetic compounds have been screened and selected for the development of insect repellents against biting insects. Insect repellents have been applied on skin of humans/animals, released as vapors into the areas around humans and animals through different delivery methods with a variety of formulations, or used to treat clothing, bed nets, and curtains. The purpose of insect repellents is to repel or reduce the contact opportunity between biting insects and humans/animals to protect the hosts from insect bites. However, most insect repellent products have not been evaluated for other activities, such as attractant, anti-oviposition, larviciding, adulticiding, synergistic and adjuvant effects against biting insects, and other use.

Since the 1940s, the materials including essential oils, plant extracts, and chemical compounds have been screened and selected for attractant, repellency, and insecticides. In the early 1960s, a report about butoxypropanediol polymer repellent showed attractant activity, when applied at a low concentration.[1] Later, through the development of new test methods and additional repellent evaluations, deet (*N*,*N*-diethyl-meta-toluamide) was also shown to provide anti-oviposition activity.[2] Since the 1990s, many natural repellents and chemical compounds were screened for anti-oviposition, larviciding or adulticiding, and other activities.

ATTRACTANTS

In 1961, Hocking first reported that vapors of butoxypolypropylene glycol (butoxypropanediol polymer) acted as an attractant to *Aedes aegypti* (L.) in a T-tube olfactometer.[1] Since the 1970s and 1980s, several scientists[3] reported that repellents, such as deet, benzimine (*N*-benzoylhexamethylenimine), rebemid (*N,N*-diethylbenzamide), and cyclopentanone-2-carboxylic acid were attractants to *A. aegypti*. Also, field studies showed that deet, benzimine, and dimethyl phthalate repellents were attractants to several species of mosquitoes, such as *Aedes cinereus* Meigen, *Aedes vexans* (Meigen), *Aedes caspius* (Pallas), *Culex modestus* Ficalbi, *Anopheles hyrcanus* (Pallas), and *Coquillettidia richiardii* (Ficalbi) and suggested that the repellents should be washed off the skin when their repellent action ceases.[4] The documents confirmed that these compounds acted as attractants at low doses and as repellents at high doses in the laboratory trials with several repellents including deet.[5]

Mehr et al.[5] reported that deet and ethyl hexanediol acted as attractants when applied at low concentrations, deposits, or residues against *A. aegypti* and *A. taeniorhynchus* Wied, but not against *Anopheles albimanus* Wied. Based on their results and other previous studies, a model sequence of the effects of compounds on mosquitoes with increasing dose was developed as (Neutral → Attractant)N → Neutral → Repellent → Toxic, where the term "repellent" includes both repellent and anti-feed effects. Thus, the U.S. Environmental Protection Agency recommended using labels that included instructions of washing off or reapplying the repellent when it is no longer effective, or washing treated skin with soap and water after and between applications.

However, so far, no insect repellents have been tested or applied at low doses as attractants in combination with any kind of traps to attract insects including mosquitoes and other biting insects. In recent studies, a natural repellent (plant oil) (undisclosed) at low concentration, which demonstrated attractant effect and stomach toxic, is combined and developed for attractive toxic sugar baits against insects (unpublished data).

ANTI-OVIPOSITION

Oviposition is a major part of the life cycle of insects. If oviposition is prevented, the life cycle of insects is disrupted and population growth may be reduced. Oviposition repellents could be used to shield the number of mosquito and other biting fly oviposition sites against gravid females that may be induced to oviposit in lethal ovitraps. As a component of the integrated approach to mosquito population management, the breeding sites could be treated with effective oviposition repellents.

Bar-Zeev and Ben-Tamar[2] used cloth treated with insect repellent to test for anti-ovipositional activity against mosquitoes in a laboratory; however, their objective was to assess anti-ovipositional activity of the chemicals against mosquitoes as a simple technique for repellent bioassay. Kuthiala et al.[6] used electrophysiological assay methods to show that the repellent deet combined with ethyl propionate reduced oviposition response against *Aedes aegypti*. Bentley and Day[7] reviewed mosquito oviposition ecology and discussed the repellency of certain insecticides and other natural products that deterred oviposition. Table 21.1 and Table 21.2 list other studies and reports on oviposition repellents, anti-oviposition compounds, or oviposition deterrents. The materials included extracts and oils from natural plant-based and synthetic chemicals.

Based on the studies of Xue et al.,[8] topical repellent deet and experimental repellents, 1-(3-cyclohexen-1-ylcarbonyl)-2-methyl-piperidine (AI3-37220) and 1-(3-cyclohexen-1-ylcarbonyl)-piperidine (AI3-35765), acted as oviposition repellents against mosquitoes at very low concentration (<1%) and provided effective anti-oviposition from egg-laying *Aedes albopictus* Skuse in the laboratory and field that lasted for 2–4 weeks. After 2–3 weeks of anti-oviposition by these insect repellents, the material in the water still killed the first instar larvae of mosquitoes after hatching. The application

TABLE 21.1 Selected Insect Repellents and Their Efficacy against Oviposition by Gravid Female *Aedes albopictus* in the Laboratory

Repellent Codes	Name of Compounds	Efficacy (ED_{50} in % or mg/L)
Deet	*N,N*-Diethyl-3-methylbenzamide	0.01
AI3-35765	1-(3-Cyclohexen1-ylcarbonyl)-piperidine	0.008
AI-37220	1-(3-Cyclohexen-1-ylcarbonyl)-2-methyl-piperidine	0.004
AI3-262	Dimethyl phthalate	0.02
AI3-14823	2-(2-Butoxyethoxy) ethylester carbamic-acid	0.01
AI3-54995	*N*-Ethyl, *N*-isopropyl-2-thiophenecarboxamide	0.008
AI3-55004	*N*-Methyl, *N*-(2-methylpropyl)-3-cyclohexenecarboxamide	0.02
AI3-55007	*N*-Methyl, *N*-(2-methylpropyl)-2-thiophenecarboxamide	0.02
AI3-55046	*N*-(1-Methylpyrrole-2-carbonyl)-diethylamine	0.01
AI3-55051	3-Methyl, *N,N*-diethyl-2-thiophenecarboxamide	0.03
AI3-55054	3-Methyl, *N*-ethyl, *N*-methyl-2-thiophenecarboxamide	0.007
AI3-55061	1-((2-Methyl-furan-3-furan) carbonyl)-azepine	0.03
AI3-55062	2,3,6-Trihydro, 1-((2-methyl-3-furyl) carbonyl)-pyridine	0.02
AI3-55127	1-(3-Furancarbonyl)-2-methyl-piperidine	0.08
AI3-55063	2-Methyl, *N*-isobutyl, *N*-methyl-3-furanecarboxamide	0.005
AI3-55120	*N*-Ethyl-*N*-(3-methoxypropyl)-cyclopropanecarboxamide	0.05
AI3-61455	*N, N′,N′*-Methylidynetris-formamide	0.05
AI3-63244	1-(2,2,3,3,3-Pentafluoro-1-oxypropyl)-pyrrolidine	0.01
AI3-63333	*N*-(3-(Dimethyl amino) propyl-2,2,3,3,4,4,4,4-heptafluoro-batanamide	0.05
AI3-64210	Hexahydro-α-methyl-1H-azepineethanol	0.05
AI3-70620	*N,N*-Diethyl-3-furancarboxamide	0.05

TABLE 21.2 Twenty-One Insect Repellents and Their Efficacy (% Mortality at 0.1% Rate at 24 Hours Posttreatment) against Early Fourth Instar Larvae and Effective Repellency (%) against Oviposition of *Aedes albopictus* in the Laboratory

Insect Repellents	Major Active Ingredient	Anti-Oviposition (%)	Mean Mortality (%)
Alfresco	Lavandula and other anti-insect moisturizer with botanical extracts	100	57
Ballet	Eucalyptus, geranium oil, citronella oil	98	88
Bite Blocker	Soybean oil, geranium oil	100	100
Bygone	Eucalyptus oil, peppermint, geranium	92	35
GonE	Eucalyptus oil, lavender oil, peppermint oil, and geranium oil	76	5
MosquitoSafe	Geraniol oil	99	100
Natrapel	Citronella	100	97
Neem Aura	Neem, cireonella, lavender, lemongrass	88	100
Quwenling	Extracts of lemon eucalyptus plant	100	92
Sketolene	Natural products	96	95
Skin-So-Soft	Citronella oil	80	97
SunSwat	Citronella oil, lavender lemon peel	100	100
Amway	31.58% Deet	94	93
OFF! Skintastic	6.65% Deet	84	90
OFF! Skintastic with sunscreen	9.5% Deet	89	57
Cutter	9.5% Deet	95	88
Sawyer		96	100
Vaseline repellent		100	87
Autan		98	97
Repel permanone	0.5% Permethrin	100	100
Skin-So-Soft	7.5% IR3535	85	100

rate of any repellent against oviposition was much less than that on the skin of animals and humans. The duration of the protection from oviposition was much longer than that of repellents on skin of humans and animals due to body temperature and humidity.[2]

LARVICIDING

More than 25,000 compounds (both natural and synthetic) were evaluated as repellents against mosquitoes.[9,10] Of these, over 2800 new compounds were evaluated since 1964 as skin repellents against mosquitoes and other biting arthropods at the U.S. Department of Agriculture's Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, Florida.[11] Xue et al.[12] reported that the selected insect repellents, deet, AI3-37220, and AI3-35765 as anti-oviposition repellents, were effective against container-breeding mosquitoes and killed their larvae. Consequently, appropriate laboratory studies on the acute toxicity of these repellents shown in Table 21.2 worked as larvicides against the larvae evaluated in the laboratory and field.

The botanical insect repellents, cinnamon oil, lemon eucalyptus oil, sandalwood oil, and turmeric oil, were evaluated in the laboratory against fourth instars of *Aedes aegypti, Aedes albopictus,* and *Culex pipiens* complex. The sandalwood oil was the most effective larvicide, killing the larvae of all three species of mosquitoes in a relatively short time.[13] Twenty-one commercial insect repellents shown in Table 21.3 including 12 natural, 6 deet-based, and 3 synthetic repellent products were evaluated as larvicides against the larvae of *Aedes albopictus* in the laboratory. Ten of the 12 botanical products at 0.1% concentration provided 57%–100% mortality at 24 hours posttreatment.[14,15]

Usually, plant oils and extracts are screened and selected for larvicidal, repellent, and adulticidal activity in the laboratory. Most of the essential oils for repellents showed the function of killing larvae and adult mosquitoes with a much higher application rate than the insecticides. All extracts from the leaves of *Eclipta alba*, *Andrographis paniculata*, *Ervatamia coronaria*, *Caesalpinia pulcherrima*, and *Citrus sinensis* orange peel showed different larviciding, adulticiding, and repellent properties against *Anopheles stephensi* Liston, *Aedes aegypti*, and *Culex quinquefasciatus* Say.[16] Fourteen repellent compounds including three experimental repellents (A13-37220, (1*S*,2*S*′)-2-methylpiperidinyl-3-cyclohexen-1-carboxamide [SS-220], and A13-35765) were evaluated against

TABLE 21.3 Selected Insect Repellents and Their Efficacy (LC_{50} in % or mg/L at 24 Hours Posttreatment) against Early Fourth Instar Mosquito Larvae in the Laboratory

Repellent Codes	*Anopheles quadrimaculatus*	*Aedes aegypti*	*Culex quinquefasciatus*
Deet	0.02 (*Anopheles albimanus*)	0.03 (*Aedes albopictus*)	N/A
AI3-35765	0.02 (*Anopheles albimanus*)	0.01 (*Aedes albopictus*)	N/A
AI-37220	0.02 (*Anopheles albimanus*)	0.02 (*Aedes albopictus*)	N/A
AI3-54995	0.1	0.1	0.1
AI3-55004	1.0	0.2	1.0
AI3-55007	1.0	0.1	0.1
AI3-55051	0.1	1.0	0.1
AI3-55054	0.1	0.1	0.1
AI3-55061	0.1	0.1	0.1
AI3-55062	1.0	0.1	1.0
AI3-55063	0.1	0.1	0.1
AI3-61455	1.0	1.0	1.0
AI3-64210	0.1	1.0	1.0
AI3-70620	0.1	0.1	0.1

Note: N/A, not available.

three species of mosquito larvae, *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles quadrimaculatus* Say, in the laboratory. Larval mortality data at 24 and 48 hours after treatment indicated that 15 test repellent compounds provided larval mortalities in the range of 67%–100% against the mosquito larvae. A multi-way analysis of variance of these data showed that the repellent compounds, concentrations used, species of mosquitoes, larval stages used, and exposure times did affect the degree of larval mortalities.[17]

ADULTICIDING

Xue et al.[18] reported that 16 commercial insect repellents (6 botanical and 10 synthetic organic products) in spray formulations produced significant adult knockdown (KD) and 24-hour mortality against laboratory-reared female mosquitoes, *Aedes aegypti, Aedes albopictus,* and *Anopheles quadrimaculatus.* The synthetic organic repellents induced faster KD than most botanical repellents and some repellents were suggested to be used as toxicants for mosquito control in some situations. Pridgeon et al.[19] reported that some insect repellents showed a high insecticidal activity against adult mosquitoes, *Anopheles albimanus*, *Anopheles quadrimaculatus, Aedes aegypti,* and *Culex quinquefasciatus*, in the laboratory. Licciardi et al.[20] reported that deet had higher insecticidal activity than 3-(*N*-butyl-*N*-acetyl)-aminopropionic acid, ethyl ester (IR3535), and KBR 3023 (Picaridin) against the adult mosquito *Aedes aegypti* by using the flight response in the laboratory assays. Different mosquito species showed different susceptibility to similar or different insect repellents. *Anopheles albimanus* was the most susceptible mosquito species to repellent toxicants.[21]

Botanical insect repellent products, MosquitoSafe (25% geraniol oil) and SunSwart, were the most effective in causing mortality of mosquito adults. Most botanical insect repellent products tested by Xue et al.[18] showed a different mortality of adult mosquitoes at 24 hours posttreatment. The species of mosquitoes used and the insect repellent products tested in the laboratory resulted in different mortality of *Aedes, Culex*, and *Anopheles* mosquitoes.[18]

Deet repellent (10% in ethanol) applied on guinea pig skin against caged *Anopheles quadrimaculatus* exposed to the treated skin for 5 minutes resulted in 98% mortality in mosquitoes after 24 hours. Also, the deet treatment on animal skin extended female mosquito's probing time and reduced blood-feeding rate and blood-engorgement duration. This study suggested that repeated exposure of female mosquito populations to deet repellent, in laboratory bioassay, could result in confounding of toxicant and repellent effects and inaccurate estimates of deet repellency (Table 21.4).[22]

SPATIAL REPELLENT EFFECT

Burning mosquito coils containing insecticides generate smoke indoors that can effectively reduce contact between host-seeking mosquitoes and humans. Most mosquito coil products contain pyrethroid active ingredients and provide personal protection by repelling mosquitoes against their bites. There are several reports that discuss the evaluations of mosquito coils' insecticidal activity; however, the ones containing natural oils did not show a strong insecticidal activity. Xue et al.[21] reported that two pyrethroid mosquito coils containing 0.08% meperfluthrin showed the strongest insecticidal activity, and other pyrethroid mosquito coils produced significantly higher mortality than the citronella coil against *Anopheles albimanus, Aedes albopictus*, and *Culex quinquefasciatus*.

Other documented studies have shown that pyrethroid insecticides provided different degrees of repellency against oviposition and mosquito bites.[7] Permethrin-treated uniforms and bed nets have also been used to repel biting insects (Table 21.5).[15]

TABLE 21.4 Sixteen Insect Repellents in Aerosol Sprays and Their Efficacy (% Mortality at 24 Hours Posttreatment) against Three Species of Adult Mosquitoes in the Laboratory

Repellent Names	Major Active Ingredients	*Anopheles quadrimaculatus*	*Aedes aegypti*	*Aedes albopictus*
Bug out	14.25% Deet	100	100	100
Cutter	9.5% Deet	100	100	100
Eckerd	14.25% Deet	100	100	100
Repel	14.25% Deet	100	100	100
Ultra Muskol	38% Deet	100	100	100
OFF! Deep Wood	23.8% Deet	100	100	100
OFF! Skintastic	6.65% Deet	100	100	100
OFF! unscented	14.25% Deet	100	100	100
Autan	Hydroxyethyl butyl piperidine, carboxylate	100	100	100
Repel permanone	0.5% Permethrin	100	100	100
GonE	Lavender oil, eucalyptus oil, soybean oil	100	100	100
MosquitoSafe	25% Geraniol	100	100	100
Natrapel	10% Citronella	100	100	75
Neem Aura	Neem oil, citronella oil, lavender oil	80	80	100
Skin-So-Soft	0.1% Citronella oil	100	90	100
SunSwart	Palm oil, citronella oil, lavender oil, lemon peel	100	100	100
Control	10% Alcohol	2	5	2

TABLE 21.5 Selected Insect Repellents and Their Efficacy (ED_{50} in % or mg/L at 24 Hours Posttreatment) against Adult Female Mosquitoes in the Laboratory

Repellent Products	Active Ingredients	*Aedes aegypti*	*Aedes albopictus*	*Anopheles quadrimaculatus*	*Anopheles albimanus*	*Culex quinquefasciatus*
OFF!, Cutter, Repel, Eckerd, Muskol, Bug out	6.65%–38% Deet	0.9	0.9	0.4	0.2	0.6
IR3535	3-*(N*-Butyl-*N*-acetyl-aminopropionic acid, ethyl ester	1.9	28	2.4	1.7	3.6
AI-37220	1-(3-Cyclohexen-1-ylcarbonyl)-2-methylpiperidine	0.3	21	0.2	0.1	0.2
DMP	Dimethyl phthalate	5.4	—	2.5	1.8	4.7
KBR3023	Picaridin	1.1	—	0.6	0.5	1.6
AI3-35765	1-(3-Cyclohexen-1-ylcarbonyl) piperidine	0.3	—	0.5	0.2	0.5
Ethyl hexanediol	2-Ethyl-1,3-hexanediol	2.9	—	1.7	1.5	1.6
PMD	*Para*-menthane-3,8-diol	1.9	—	1.5	1.3	2.1

SYNERGISTS AND ADJUVANTS

Recently, a new long-lasting repellent-treated bed net has been impregnated with the skin repellent, deet or IR3535, onto the fibers of mosquito bed net fabric using a new polymer-coating method. Both deet- and IR3535-impregnated fabrics showed a dose-dependent insecticidal activity and resulted in 100% KD from 187 to 28 minutes against *Aedes aegypti*, whereas the one with IR3535 was from 88 to 58 minutes. This method is highly promising as a potential candidate for future malaria control strategies, particularly in areas where pyrethroid resistance occurs.[23,24]

The primary author conducted an experiment with deet mixed with permethrin insecticide against *Aedes aegypti* and showed that the mixture knocked down the adult mosquitoes faster than permethrin in the laboratory bioassay. This preliminary finding may help us to use a mixture of deet with other insecticides to combat the resistant strain of mosquitoes in the future (Xue, unpublished data). The mode of action, mechanism, and practice are still needed to be further studied in the future.

CONCLUSION

Several synthetic insect repellents showed attractance when they were applied using low concentrations. Both plant-based and synthetic insect repellents showed effective anti-oviposition at relatively low concentrations against gravid female mosquitoes.[8] In addition, the persistence for anti-oviposition was much longer than the duration of protection on human and animal skin from biting insects, usually lasting for few weeks in the laboratory and field. The female mosquitoes did not lay their eggs and retained them in their bodies when they visited water treated by insect repellents because the duration of egg retention reduced the egg reproduction and hatching.[25] Also, Xue et al.[26] showed that the insect repellents used as anti-oviposition for mosquitoes were safe and did not have a significantly negative effect on other non-target aquatic organisms in the laboratory bioassay. For specific containers and some breeding sites, these insect repellents may be used to prevent mosquito egg laying.

Most insect repellent products including essential oils and extract materials from plant-based[27–29] and synthetic chemical compounds showed a considerable degree of larviciding and adulticiding activity against mosquito larvae and adults evaluated in the laboratory. Most synthetic spatial repellents showed a strong adulticiding activity regardless of formulations. Some insect repellents combined with other insecticides showed increasing and additional effectiveness against mosquitoes and other biting insects. The synergistic and adjuvant effects of insect repellents with other mode of action of insecticides may be beneficial against resistant biting insects. The anti-oviposition and insecticidal activity of insect repellents have the potential to be used as some of the ideal approaches for the management of mosquitoes and other biting insects.

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CHAPTER 22

Future of Insect Repellents

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The *Insect Repellents Handbook* reviews the current state of science and product development for personal protection from biting arthropods. The world currently enjoys a wide variety of tools, including a variety of topical repellents, protective clothing, area repellents, bed nets, and screens. Despite a relatively small commercial market, vigorous improvement of these products continues. Manufacturers seek new active ingredients and new formats for putting those chemicals between a person and an arthropod. Applied research supports those improvements and also provides realistic estimates of the effectiveness of personal protection. Basic research makes scientific discoveries that are the sources of the truly innovative solutions of the future, either by discovering the unexpected or by careful description of biological mechanisms.

The performance of topical repellents has usually been judged based on duration of essentially complete protection. Manufacturers are concerned with sales that depend not only on performance but also on user acceptability. Therefore, the elements of a topical repellent might be considered as active ingredient, formulation, and packaging. Active ingredients influence how many different kinds of biting arthropods are repelled, how thoroughly they are repelled, and the duration of protection. The formulation also influences duration but, perhaps more importantly, largely determines application characteristics of that particular active ingredient. Packaging contributes to how the product is used and can make a big difference in whether the repellent is convenient.

N,N-diethyl-3-methylbenzamide (deet) has set the standard for performance of an active ingredient, raising the expectation of complete protection from any biting arthropod, with duration depending on concentration and formulation. New active ingredients, such as picaridin or 2-undecanone, have contributed incremental improvements over deet in performance and acceptability. Many other potential active ingredients have been described but not marketed and the best ones are all comparable to deet. Laboratory findings of two- or threefold improvements in duration of some new active ingredients may not be significant improvements over deet, if those new chemicals ever find their way into products. The current state of active ingredient discovery leaves the impression that performance of deet, picaridin, IR3535, and *p*-menthane-3,8-diol (PMD) is similar and that new active ingredients in the laboratory only provide variation in the spectrum of activity without making a major change. In other words, there exists a wide variety of active ingredients that have different advantages and disadvantages, but they are all likely to result in topical repellent products that require on the order of 20% concentration of active ingredient to provide 4–8 hours of nearly complete protection. Given this armamentarium of active ingredients, consumers have important

choices to make, but they will find that the actual use of the various repellent active ingredients is similar.

Formulation has come a long way since 75% deet in alcohol was the standard U.S. military repellent. Systematic efforts to extend duration by mixing deet into polymers resulted in a product that contained only 33% deet and that provided protection for 12 hours. Part of the successful performance of that product, which is still on the market, was because of its thick consistency that resulted in a greater dose of active ingredient on the skin. The same toothpaste-like consistency makes the product unpleasant to use. More advanced formulations that take advantage of microencapsulation achieve similar duration with thinner, easier-to-apply products that have the added advantage of limiting absorption of deet through the skin. Duration of protection is important, and new labeling requirements by the U.S. Environmental Protection Agency (EPA) emphasize comparison based on duration against ticks or mosquitoes. However, the consumer is probably just as influenced by application characteristics, including color, skin feel, and odor. In some cases, people probably identify the "strength" of a product with its chemical odor and might actually seek a product with a strong smell. Presumably, most people want something that either has no odor or has a pleasant odor. This is an area where active ingredient can make a big difference, as deet requires considerable formulation to hide its odor. Picaridin and PMD have less odor and to some observers, more pleasant. Only IR3535 has virtually no odor, which may be part of the success of those products containing this active ingredient. Active ingredients can also make a big difference in skin feel. Deet is very oily and because it is usually used at a concentration of at least 20%, formulators have a difficult task to modify their products. Although a low percentage product, adsorption on corn starch results in a pleasant product and the various microencapsulated formulations also improve skin feel. Botanical products generally have less effectiveness, but their odor and skin feel usually appeal to the consumer. Part of that appeal might be from being accustomed to using those same scents in many skin care products and another part might be the association of those odors with food or cleanliness. At least one botanical product with qualities very similar to a skin cream has been reported to achieve excellent effectiveness, though that product has not appeared on the market in a major way. Specialty formulations are useful to smaller segments of the market, for example combinations of sunscreen and repellent, products formulated in stick form, camouflage face paint with repellent, towelettes, and so on.

Packaging can make a large difference in whether or not a product is used and influences the shelf life of the product. The excellent stability of deet has probably fixed the idea in consumers' and retailers' minds that a bottle of repellent should last from year to year. Although such stability is convenient, the expectation that all products will last indefinitely could be unrealistic. Manufacturers sometimes use outer packaging to extend shelf life, as well as prevent tampering. The consumer throws the outer packaging away, but he or she must deal with the product itself every time it is used. A runny liquid in an open-topped bottle is not convenient. A pump spray with a spring that ceases to function can waste an entire bottle of repellent. Tubes with sharp corners that tear open plastic bags are an annoyance. In fact, there are many ways that packaging can detract from the qualities of an otherwise useful product. Some of the innovations that make sense are external caps that provide a tight seal against leakage, brightly colored containers that are easy to find, and even tubes with clips so that the product is always handy for use. Although not strictly a part of packaging, the label should also be important. The new U.S. EPA requirement for a very specific symbol indicating hours of duration against ticks and mosquitoes will certainly communicate a clear message. Other directions in fine print with various caveats and warnings are perhaps less useful, but provide the legal (in the United States) definition of proper use, relieving the manufacturer from many liabilities.

Area repellents appeal to consumers for several reasons. First, they are easy to use, especially if several people are gathered together. Second, they do not require skin application. Finally, their use often corresponds to other daily customs, for example, lighting candles. Unfortunately,

good efficacy of area repellents is difficult to achieve. The inherent difficulty is that any technique that depends on dispersal of a chemical into the air will be less effective when air is moving—and air tends to move outdoors. The most successful products have used actively dispersed pyrethroids. Indoors, they can probably provide nearly complete protection, but outdoors, their effectiveness may be reduced by half or worse. New active ingredients under development show some promise based on either enhanced volatility or completely new modes of action that disrupt insect behavior. As with all repellents, the use of area repellents against ticks has lagged far behind their use against flying insects. The idea of a material that could be applied to the ground and prevent tick bites could be important for disease prevention, but no such material exists yet. Area repellent systems based on physics rather than chemistry have been proposed, manufactured, and sold in bulk. Unfortunately, none of the sound-producing or electromagnetic products have had any effect on biting arthropods. Nonetheless, mosquitoes respond to sound and to magnetic fields, so that the biological basis for future products may exist.

Barriers such as screens, doors, windows, roofs, and clothing are very important for preventing bites. Much of the construction that we take for granted has the primary or secondary purpose of preventing entry of insects. The technology for window and door screens is finely developed, practical, and cheap. Clothing provides another kind of barrier, especially when it is loose fitting or when it is worn in double layers. Permethrin treatment of clothing is especially effective against ticks and chiggers, but it has the disadvantage of not protecting adjacent skin. New active ingredients for clothing treatment may provide more protection, but they will at least provide alternatives that improve effectiveness against resistant arthropods. Very recent work on the design of weave patterns that completely prevent biting by mosquitoes while allowing good air flow on the skin introduces an exciting new possibility for protection against bites.

Based on the current state of product development, the possibilities introduced by biology, and a focus on consumer need, what products might we hope to see developed in the next generation? For topical repellents, we can expect the discovery of new active ingredients that break the deet paradigm and provide much greater inherent protection. A chemical that was active at pharmaceutical levels against biting arthropods might be up to 10,000 times more effective than deet. Such a chemical could be used at a low percentage, creating the possibility of ideal formulations that are easy to use. Highly effective chemicals might be more specific than the broad spectrum repellents we use now, which would make it necessary to make more informed choices of repellent products. Recent discoveries about the nature of olfactory and gustatory reception have introduced repellent science to completely new modes of action. In addition to perhaps guiding the search for more effective topical active ingredients, those discoveries also may lead to more effective area repellents. The current development of compounds that block mosquitoes' ability to detect odors is one promising example. Given the public's obvious desire for repellent candles, torches, coils, and other devices, highly effective area repellent active ingredients would find a vigorous market. Screens and construction may be mature technologies, but protective clothing is developing rapidly. The idea of nonchemical barriers created by special weaves is appealing because it would not involve any of the complications of a pesticide and possibly not cost much more than the manufacture of normal textiles.

We would like to see a future in which daily use products such as deodorant and toothpaste also protect the user from biting arthropods and where area repellent products provide complete protection reliably in the situations where they need to be used. For the last 70 years, since the formulation of the first modern topical repellents, we have only seen one real paradigm shift when deet was introduced in the 1950s. Incremental improvements are welcome, but recent concentration on the physiology of insect chemical reception and a philanthropic desire to protect billions of people from malaria and dengue may be the ingredients for another paradigm shift based on chemical discovery. As a practical matter, we can only hope that scientific discovery is matched by practical development that results in true personal protection from arthropod bites.

Appendix

Table A1.1 Identification of Known Repellent and Insecticide Active Ingredients

Common Name	Common Chemical Name	Formula Name	CAS No.
2-Butyl-2-ethyl-1,3-propanediol			115-84-4
A			
Acepromazine		1-[10-(3-Dimethylaminopropyl) phenothiazine-2-yl] ethanone	61-00-7
Acetaminophen		*N*-(4-hydroxyphenyl)ethanamide	103-90-2
Acetic acid	Ethanoic acid	Methanecarboxylic acid, Acetyl hydroxide (AcOH), Hydrogen acetate (HAc)	64-19-7
AI3-35765	1-Cyclohex-3-enyl-(1-piperidyl) methanone	1-[3-Cyclohexen-1-ylcarbonyl] piperidine	52736-58-0
AI3-37220 (SS220)	1-Cyclohex-3-enyl-(2-methyl-1-piperidyl) methanone	1-[3-Cyclohexen-1-ylcarbonyl]-2-methylpiperidine	77251-47-9
Allethrin		(2-Methyl-1-propenyl)-2-methyl-4-oxo-3-(2-propenyl)-2-cyclo-penten-1-ylester or mixture of *cis* and *trans* isomers	584-79-2
α-Cypermethrin		*rel*-(*R*)-cyano-(3-phenoxyphenyl) methyl(1*S*,3*S*)-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate	67375-30-8
Alphamethrin		[Cyano-(3-phenoxyphenyl)-methyl] 3 (2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate	97955-44-7
B			
β-alanine	β-Alanine	3-Aminopropanoic acid	107-95-9
Benzyl benzoate		Phenylmethyl benzoate	120-51-4
Butane			106-97-8
β-Cyfluthrin		[Cyano-(4-fluoro-3-phenoxy-phenyl)-methyl] 3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropane-1-carboxylate	68359-37-5
BHT	Dibutyl hydroxyl toluene	4-Methyl-2,6-ditert-butyl-pheno	128-37-0
BHC	Benzene hexachloride (See Lindane)	1,2,3,4,5,6-Hexachlorocyclohexane	608-73-1

(*Continued*)

Table A1.1 Identification of Known Repellent and Insecticide Active Ingredients (*Continued*)

Common Name	Common Chemical Name	Formula Name	CAS No.
C			
Camphor		1,7,7-Trimethylnorbornan-2-one	76-22-2
Citronella		3,7-Dimethyloct-6-enal	26489-02-1
Citronellal		3,7-Dimethyloct-6-enal	106-23-0
Citronellol		3,7-Dimethyloct-6-en-1-ol	106-22-9
Citric acid		2-Hydroxypropane-1,2,3-tricarboxylic acid	77-92-9
Carbon dioxide			124-38-9
D			
DBP	Dibutyl phthalate	Dibutyl benzene-1,2-dicarboxylate	84-74-2
DMP	Dimethyl phthalate	Dimethyl 1,2-benzenedicarboxylate	131-11-3
Deet	Diethyl toluamide	*N*,*N*-diethyl-3-methylbenzamide, *N*,*N*-diethyl-*m*-toluamide	134-62-3
DEPA	*N*,*N*-diethyl-2-phenylethanamide	*N*,*N*-diethyl phenyl acetamide	2431-96-1
Dehydrolinalool		3,7-Dimethyl-6-octen-1-yl-3-ol	Not available
Deltamethrin		[Cyano-(3-phenoxyphenyl)-methyl]-3-(2,2-dibromoethenyl)-2,2-dimethyl-cyclopropane-1-carboxylate	62229-77-0
DMC	Dimethyl carbate	Dimethyl bicyclo[2.2.1] hept-2-ene-5,6-dicarboxylate	5826-73-3
Dioctyl phthalate		Bis(2-ethylhexyl) benzene-1,2-dicarboxylate	117-81-7
DDT		1-Chloro-4-[2,2,2-trichloro-1-(4-chlorophenyl)-ethyl]-benzene	50-29-3
d-trans-Allethrin		(2-Methyl-4-oxo-3-prop-2-enyl-1-cyclopent-2-enyl) 2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane-1-carboxylate	28057-48-9
E			
Ethyl hexanediol	Rutgers 612	2-Ethyl-1,3-hexanediol	94-96-2
Etofenprox		1-[[2-(4-Ethoxyphenyl)-2-methylpropoxy] methyl]-3-phenoxy-benzene	80844-07-1
Esbiothrin		(2-Methyl-4-oxo-3-prop-2-enyl-1-cyclopent-2-enyl) 2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane-1-carboxylate	84030-86-4
F			
Formic acid			64-18-6
G			
γ-Octalactone		Not available	
Geraniol		3,7-Dimethylocta-2,6-dien-1-ol	106-24-1
H			
HBTX	Homobatrachotoxin		23509-17-3
Heptanoic acid			111-14-8
Hexanoic acid			142-62-1
Hexanal			66-25-1
I			
IR3535	Ethyl butyl acetyl aminopropionate	3-(*N*-acetyl-*N*-butyl) aminopropionic acid ethyl ester	

Table A1.1 Identification of Known Repellent and Insecticide Active Ingredients (*Continued*)

Common Name	Common Chemical Name	Formula Name	CAS No.
Indalone	Indalone	Butyl-3,3-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-carboxylate, 2,2-dimethyl-6-carbobutoxy-2,3-dihydro-4-pyrone	532-34-3
Isopulegol		5-Methyl-2-prop-1-en-2-yl-cyclohexan-1-ol	59905-53-2
L			
λ-Cyhalothrin		[Cyano-(4-phenoxyphenyl)-methyl] 3-(2-chloro-3,3,3-trifluoro-prop-1-enyl)-2,2-dimethyl-cyclopropane-1-carboxylate	91465-08-6
Lactic acid	L-Lactic acid	2-Hydroxypropanoic acid	79-33-4
Linalool	3,7-Dimethyl-1,6-octadien-3-ol	3,7-Dimethylocta-1,6-dien-3-ol	78-70-6
Lanolin			8040-96-8
Lindane		1,2,3,4,5,6-Hexachlorocyclohexane	58-89-9
M			
Minoxidil		3-Hydroxy-2-imino-6-(1-piperidyl) pyrimidin-4-amine	38304-91-5
MGK Repellent 11		4,5a,6,9,9a,9b-Hexahydro-1H-dibenzofuran-4a-carbaldehyde	126-15-8
MGK Repellent 326		Di-*n*-propyl isocinchomeronate	136-45-8
MGK 264			113-48-4
Metofluthrin		2,3,5,6-Tetrafluoro-4-(methoxymethyl)-phenyl]methyl 2,2-dimethyl-3-prop-1-enyl-cyclopropane-1-carboxylate	240494-70-6
N			
N-butylacetanilide		*N*-butyl-*N*-phenyl-ethanamide	91-49-6
Nonanal			124-19-6
Nepetalactone		2,7-Dimethyl-4-oxabicyclo[4.3.0] non-2-en-5-one	490-10-8
Neem	No details available		
O			
Octanal			124-13-0
Octenol		1-Octen-3-ol	3391-86-4
P			
Piperidine			110-89-4
Piperine		5-Benzo[1,3]dioxol-5-yl-1-(1-piperidyl) penta-2,4-dien-1-one	94-62-2
Pirimiphosmethyl		4-Dimethoxyphosphinothioyloxy-*N,N*-diethyl-6-Methylpyrimidin-2-amine	29232-93-7
Pilocarpine		3-Ethyl-4-[(3-methylimidazol-4-yl) methyl] oxolan-2-one	92-13-7
Picaridin	KBR 3023	1-Piperidine carboxylic acid, 2 (2-hydroxyethyl)-, 1-methylpropylester	119515-38-7
PMD	*para*-Menthane diol	*para*-Menthane-3,8,-diol, 2 (2-hydroxypropan-2-yl)-5-methyl-cyclohexan-1-ol	003564-98-5
Permethrin		[3-(Phenoxy)phenyl] methyl-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate	52645-53-1

(*Continued*)

Table A1.1 Identification of Known Repellent and Insecticide Active Ingredients (*Continued*)

Common Name	Common Chemical Name	Formula Name	CAS No.
Pyrethrin		(2-Methyl-4-oxo-3-prop-2-enyl-1-cyclopent-2-enyl) 2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane-1-carboxylate	584-79-2
Polyvinylpyrrolidone (PVP)		1-Ethenylpyrrolidin-2-one	9003-39-8
Pyridostigmine bromide		(1-Methylpyridin-5-yl) dimethylaminoformate bromide	101-26-8
Piperonyl butoxide		5-[2-(2-Butoxyethoxy)ethoxymethyl]-6-propyl-benzo [1,3] dioxole	51-03-6
Q			
Quwenling	PMD	*para*-Menthane-3,8,-diol	003564-98-5
R			
Resmethrin		5-Benzyl-3-furyl)methyl 2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane-1-carboxylate	10453-86-8
S			
Soybean oil		Not available	84776-91-0
S-bioallethrin		(2-Methyl-4-oxo-3-prop-2-enyl-1-cyclopent-2-enyl) 2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane-1-carboxylate	28434-00-6
T			
Tartaric acid		2,3-Dihydroxybutanedioic acid	87-69-4
trans-Allethrin		(2-Methyl-4-oxo-3-prop-2-enyl-1-cyclopent-2-enyl) 2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane-1-carboxylate	584-79-2
Tetramethrin		1,3-Dioxo-4,5,6,7-tetrahydroisoindol-2-yl) methyl 2,2-dimethyl-3-(2-methylprop-1-enyl) cyclopropane-1-carboxylate	7696-12-0
Thujic acid		5,5-Dimethylcyclohepta-1,3,6-triene-1-carboxylic acid	499-89-8
Transfluthrin		2,3,5,6-Tetrafluorophenyl) methyl 3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropane-1-carboxylate	118712-89-3
V			
Vanillin		4-Hydroxy-3-methoxybenzaldehyde	121-33-5
Z			
(Z)-4-decenal		Deca-4,6-dien-1-ol	21662-09-9

Source: http://www.chemindustry.com/apps/chemicals.

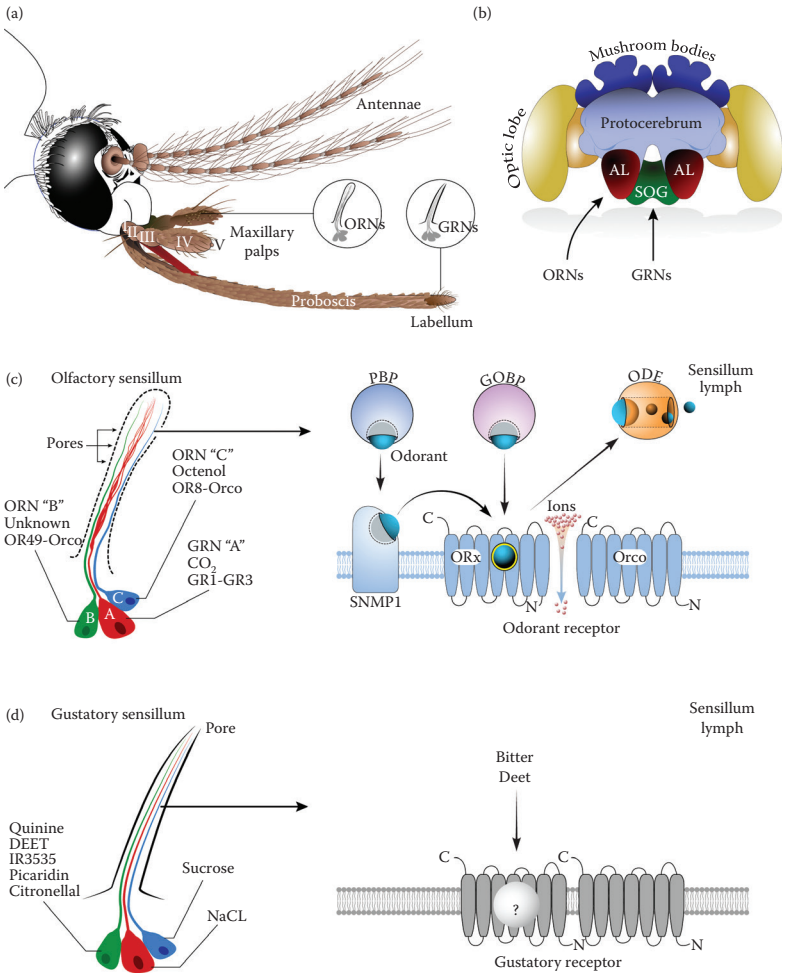


Figure 2.1 Chemical sensing in the female *Aedes aegypti* mosquito. (a) The peripheral olfactory system is distributed onto three types of appendages on the head of mosquitoes: the antennae, the maxillary palps, and at the extremity of the proboscis (labellum). (b) Chemosensory information detected by olfactory receptor neurons (ORNs) and gustatory receptor neurons (GRNs) is sent to the antennal lobe (AL) and subesophageal ganglion (SOG) in the brain. (c) Basiconic sensilla are located on the surface of the fourth segment (IV) of the maxillary palp. Multiple pores in the cuticle allow odorants to interact with ORNs. ORN "A" responds to CO_2 with the largest amplitude action potential, via the activation of at least two gustatory receptors (GR1 & GR3). ORN "B" responds to an unknown odorant with an intermediate size action potential. 1-Octen-3-ol elicits the smallest action potential from ORN "C". In *Aedes aegypti*, ORNs "B" and "C" are thought to express OR49-Orco and OR8-Orco assemblages, respectively. Accessory proteins, both soluble and membrane bound, are thought to participate in the activation of ORs. Several possible models include pheromone-binding proteins (PBPs) delivering the odorant to sensory neuron membrane protein 1 (SNMP1), which in turn offloads the odorant to the receptor. An alternative possibility is that a general odorant-binding protein (GOBP) directly transfers the odorant to the receptor. (d) Gustatory sensilla are located on the labellum and legs[76] of mosquitoes, and perhaps wing margins as shown in *Drosophila melanogaster*.[30] Gustatory sensilla located on the labellum process at least three types of compounds: salt, sweet, and bitter. The topology, stoichiometry, and potential molecular partners of GRs are not well understood.

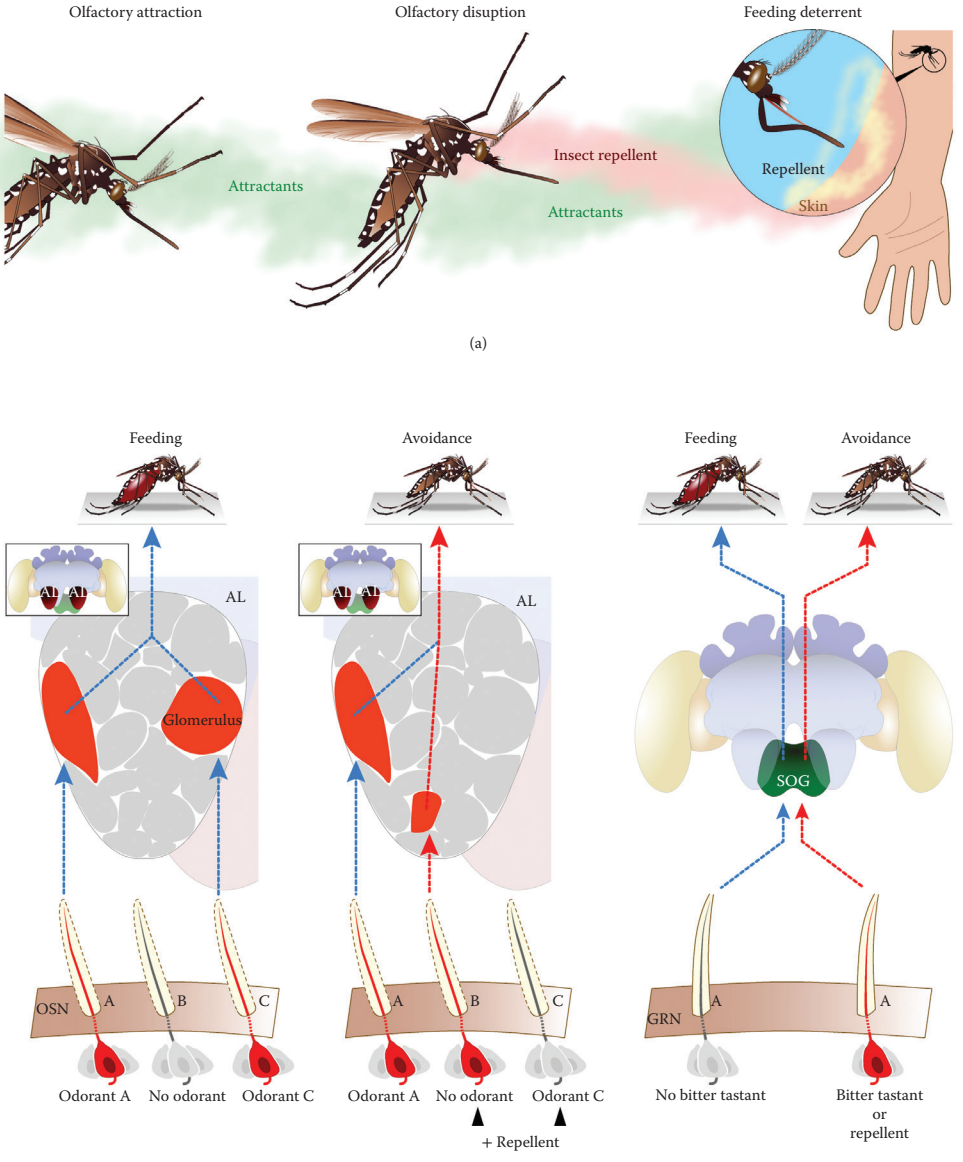


Figure 2.4 Modulation of olfactory and gustatory inputs to the brain leads to behavioral disruption. (a) Kairomones emitted by the host participate along with other sensory cues in attracting mosquitoes. At close range, this attraction will be compromised by high concentrations of insect repellents. The mosquito will go back and forth between these two states until a decision is made to quit engaging the host. On contact with the skin, insect repellents act as feeding deterrents. (b) Attraction is the result of the activity of various brain centers including the antennal lobe (AL). In the presence of attractants, odorants A and C activate olfactory receptor neurons (ORNs) A and C, respectively. The collective activity of ORNs elicits specific activation patterns of glomeruli within the AL. Insect repellents disrupt this pattern by either activation of one or multiple odorant receptors (ORs) or inhibition of other ORs. The resulting disrupted pattern of glomerular activity leads to a confused behavior. Bitter compounds and insect repellents disrupt feeding behavior by exciting gustatory receptor neurons (GRNs) located on the labellum. Sensory output from taste sensilla on the labellum first projects to the subesophageal ganglion (SOG) before reaching higher brain centers. Organotopic and functional organization of taste information in the SOG is not as well understood as in the AL. (From Isono and Morita, *Front. Cell Neurosci.*, 4, 20, 2010.)

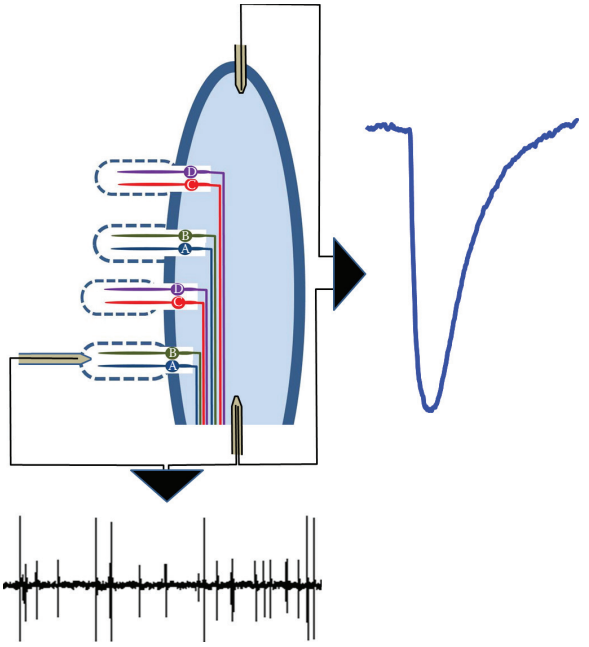


Figure 3.2 Schematic overview of insect antennal structures and possible modes of electrophysiological measurements. Antennae are adorned with many sensillum types, each housing olfactory receptor neuron (ORNs) of various sensitivities as defined by the olfactory receptors they express. Approximate summated responses from many/all sensilla can be recorded in the form of voltage deflections, termed electroantennogram (right), or individual ORN responses can be measured by penetrating a single sensillum (bottom).

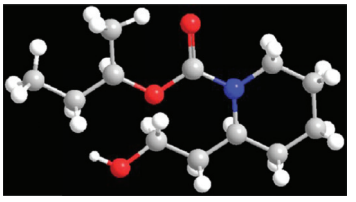


Figure 4.2 Structure of picaridin (KBR 3023).

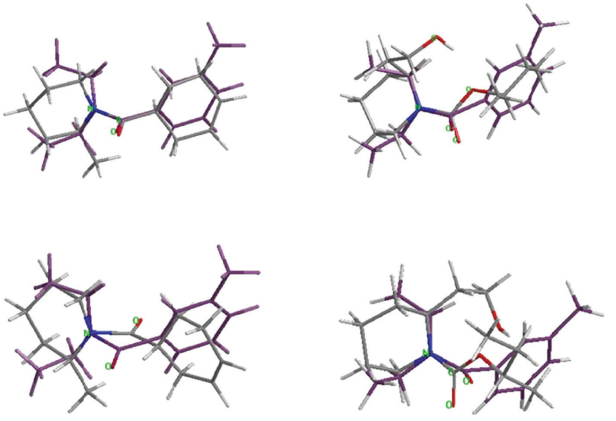
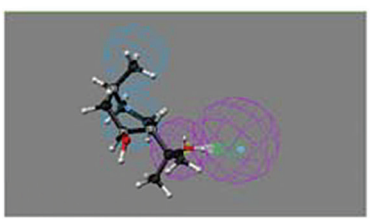
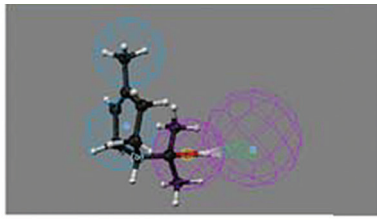


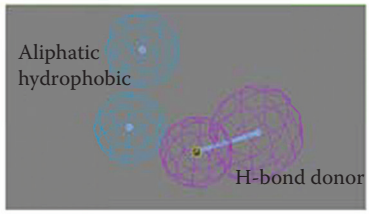
Figure 4.4 Overlay of the most active and the least active diastereomers of AI3-37220 and picaridin over deet.

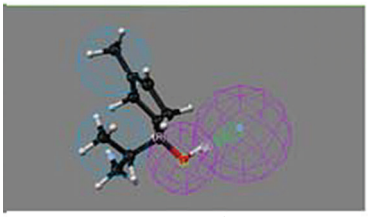


Compound 1

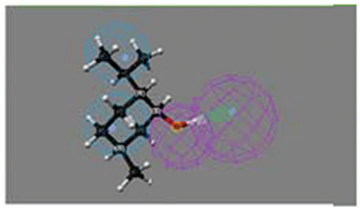


Compound 3



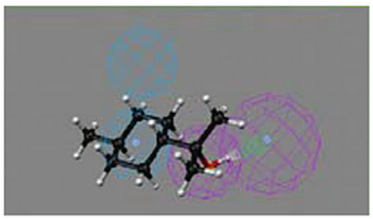


Compound 5

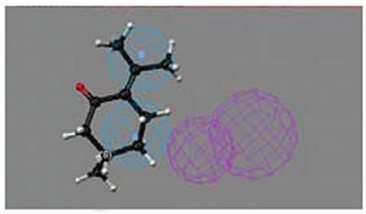


Compound 9

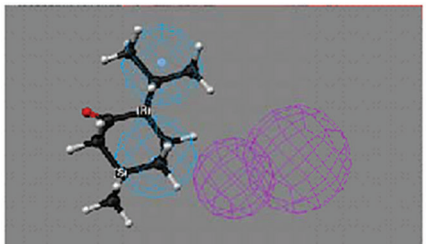
(a)



Compound 4



Compound 7



Compound 8

(b)

Figure 4.7 Pharmacophore of *p*-menthane-3,8-diol (PMD) analogs. (a) Mapping of the pharmacophore on the PMD analogs with better repellent activity. (b) Mapping of the pharmacophore on the PMD analogs with poor repellent activity.

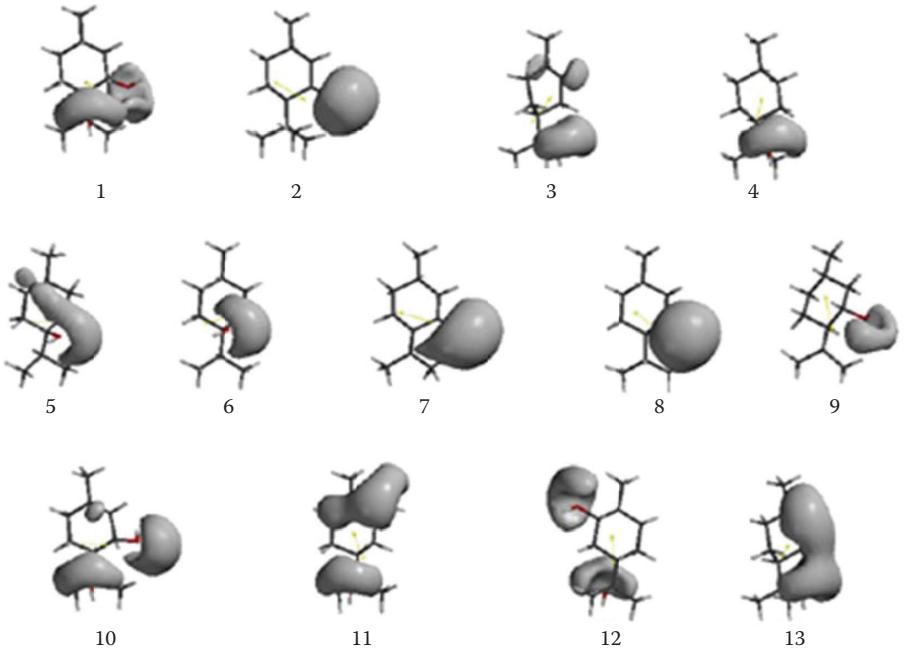


Figure 4.8 Polarity directions and molecular electrostatic potential (MEP) at 20 kcal/mol of *p*-menthane-3, 8-diol analogs.

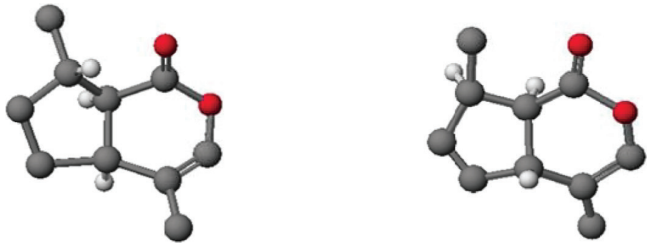


Figure 5.2 *Z,E*-nepetalactone (left) and *E,Z*-nepetalactone (right).

Figure 14.3 (a) The seated volunteer uses an ink pen and an acrylic plastic template, representing the base and 3 cm × 4 cm openings of the K & D module, to mark skin areas of his thigh to be treated with 0, 3, 6, 12, 24, and 48 nmol/cm^2 skin doses of repellent chemical against *Aedes aegypti*. (b) Shows skin areas marked for treatment. Each row of six (3 cm × 4 cm) rectangular marks running down the volunteer's leg represents where a six-celled K & D module will be positioned on the volunteer's legs. Each row represents one replicate test of six repellent doses. Six treatments for each skin area in a row are randomly assigned for application to both legs of a person and yield six randomized replicates (blocks) per volunteer (in effect, a split plot design). (c) Shows the procedure for loading each of a module's six cells with five female *Aedes aegypti* from a 1 gallon screened carton holding 5- to 15-day-old male and female mosquitoes. Mosquitoes were usually maintained with sugar-water moistened cotton balls, but were provided water only 24 hours and no water for another 24 hours before being used in a bioassay. This treatment optimized the propensity of mosquitoes to feed in the bioassay. Once a set of mosquitoes have been transferred to a module, they should be utilized in the bioassay within 45 minutes to assure maximum biting propensity. (d) Shows randomized and replicated dose treatments being applied in 55 µL ethanol to marked areas of inner, top, and outer thigh skin surfaces. In applying a treatment, the solution is applied as uniformly as possible over the 3 cm × 4 cm and about 0.5 cm outside of the rectangular marking to assure that all skin surface subsequently exposed to the insects contains test chemical. Thus, the treatment solution is applied over a 4 cm × 5 cm area (20 cm^2) of skin, but the test insect is exposed only to a 3 cm × 4 cm area of skin. As a rule for general screening tests, chemicals being tested on human skin should be applied at a rate of 24–50 nmol/cm^2 skin. In this dose range, deet suppresses mosquito biting by about 80% compared to untreated skin.[11] (e) Sliding doors of each cell of the module are opened to expose the five mosquitoes in each cell to skin below for 2 minutes. (f) Mosquitoes are shown feeding on a control area of untreated skin after a 2-minute exposure to the skin. The number of mosquitoes probing the skin surface and engorging in each cell of the K & D module is recorded. Inspection of the figure shows that four of five mosquitoes are on the skin probing and engorging. The fifth mosquito is sitting on the plastic of the cell interior. The number of insects biting (in this case, four) in a cell is recorded and then its door is then slowly closed causing the mosquitoes to leave the skin surface and fly up into the closing cell.

Figure 16.1 SPLAT Verb for protecting individual *Pinus contorta* from *Dendroctonus ponderosae* attack was applied with mechanical application equipment housed in the bed of a John Deere Gator (left, center). The same system can be adapted to a helicopter, airplane, tractor, or pickup truck. *Pinus contorta* baited with a *Dendroctonus ponderosae* tree bait (brown pouch) following application of SPLAT Verb during a pilot study (right).

Figure 16.4 Crown fade (yellow-brown needles) in untreated control trees used in SPLAT Verb pilot study. Only 6.7% of the untreated control trees were without signs of crown fade at the time when final evaluations were made (June of the field season following treatment).

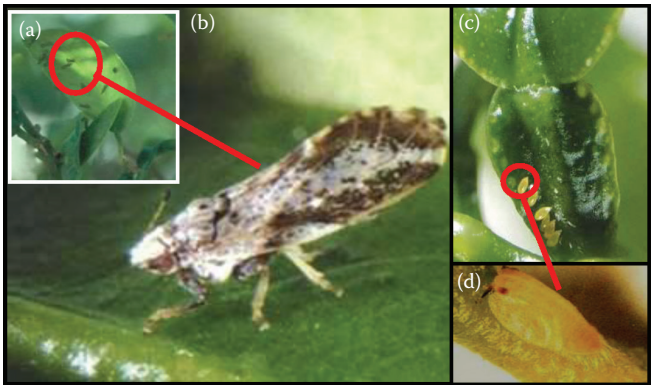


Figure 16.6 *Diaphorina citri*, Asian citrus psyllid, the vector of the *Candidatus Liberibacter* spp. that causes the devastating and irreversible Huanglongbing (HLB), or citrus greening disease. Today, Asian citrus psyllid (ACP) is present in every citrus-producing state in the United States. ACP is a very effective vector of HLB because *Candidatus Liberiobacter* grows extremely fast inside the infected nymphs (c, d), amplifying their presence and thus increasing their hosts' ability to vector HLB as adults. Thus, the effective control of *Diaphorina citri*, and keeping population at extremely low levels, is very important to citrus growers. SPLAT ACP Repel represents an important tool for the management of *Diaphorina citri* (a, b).

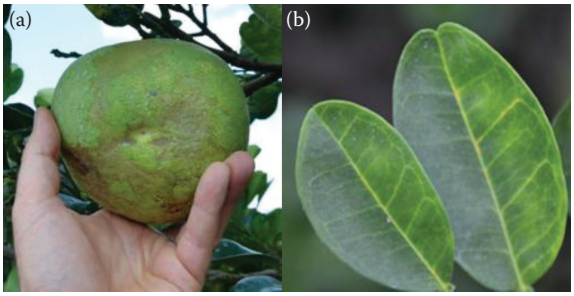


Figure 16.7 Huanglongbing (HLB) is one of the most destructive citrus diseases worldwide. (a) A pomello fruit showing classic symptoms of infection including stunted growth and an irregular shape. The fruit itself is not palatable. (b) A leaf showing the corky veins and the unbalanced chlorosis, characteristic of HLB infection.

Figure 17.1 Tom Simmons, developer of Mosbar, applies the repellent soap to the arm of an Australian soldier in 1986.

Life Science

Insect Repellents Handbook

The public has a great desire for products that prevent the annoyance of biting insects and ticks, but that desire does not always translate into sensible use of those products. **Insect Repellents Handbook, Second Edition** summarizes evidence-based information on insect repellents to inform decisions by those involved with insect repellent research, development, and use. This authoritative, single-volume reference makes it possible for the individual to gain a working level of expertise about insect repellents in a timely manner, without having to search through the literature.

The book includes a thought-provoking discussion on how repellents work, their neuromolecular basis of action, and whether green chemistry can provide effective repellents. It also supplies an in-depth understanding of the development of repellents including testing methods, review of active ingredients, and the use of chemical mixtures as repellents. It provides various science-backed chapters on repellent use including best practices for use of personal protection products, criteria for repellent use, and insect repellents for other potential use.

The previous edition was the first comprehensive volume on this subject, and it is recognized as a key reference on insect repellents. This second edition reflects the current state of insect repellent science, covers the processes involved in the development and testing of new active ingredients and formulations, and discusses the practical uses of repellents. It is a resource that will be useful to a wide variety of professionals, including insect repellent researchers, medical entomologists, public health professionals, medical personnel, industry and sales professionals, government regulators, and wildlife scientists and managers.

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